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Structure and function of bacterial communities during succession on dead plant biomass

Struktura a funkce bakteriálních společenstev v průběhu sukcese na odumřelé rostlinné biomase

Diploma thesis

Supervisor: RNDr. Petr Baldrian, Ph.D.

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Declaration:

I declare that all sources and literature are properly cited and that the content of this thesis or its major part was not previously used for obtaining of the same or other academic degree.

Prague, 12th August 2015

Bc. Vojtěch Tláskal

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Abstract

The decomposition of dead plant biomass substantially contributes to the carbon cycle and therefore is one of the key processes in temperate forests. While the role of fungi in litter and deadwood decomposition was repeatedly addressed, there are just a few surveys of bacteria associated with decomposing plant biomass. The development of bacterial community within leaf litter is likely driven by the changes in litter chemistry and by the availability of nutrients in the litter. Fungal activity greatly contributes to changing properties of substrate and thus influences bacterial community. Availability of nutrients is changing during biomass decomposition from easily accessible substrates toward more recalcitrant ones (e.g. lignin). The colonization of deadwood by bacteria is influenced by various factors such as microclimate conditions, tree species and volume. The aim of this thesis was to describe bacterial community dynamics during the first two years of decomposition of leaf litter and deadwood. In the leaf litter experiment, bacterial community was analysed in the live, senescent and decomposing leaves of *Quercus petraea*. This experiment was performed in the Xaverovsky Haj Natural Reserve, Czech Republic. Deadwood experiment was focused on the composition of bacterial community in the initial stages of decomposition of fine and coarse woody debris of *Fagus sylvatica* and *Abies alba* and was performed in the Bavarian Forest National Park, Germany. The composition of bacterial community was characterized by 16S rRNA sequencing. Enzyme activities, fungal and bacterial biomass content and loss of dry plant mass were used to explain the development of bacterial communities. Members of the classes *Alpha-*, *Beta-*, and *Gammaproteobacteria* and the phyla *Actinobacteria*, *Bacteroidetes* and *Acidobacteria* were dominant in both experiments. The genera *Sphingomonas*, *Mucilaginibacter*, *Burkholderia* and *Pedobacter* which were previously described as cellulolytic were detected. Bacteria from phyllosphere were replaced by other taxa soon after leaf senescence. Following development showed dynamic successive changes of bacterial taxa as a result of changes in substrate availability. This succession led to a diverse community at the end of leaf litter decomposition. Life strategies of early colonizers as well as late specialists were distinguished. Diameter of

decomposing wood influenced bacterial community in the first year of decomposition while tree species was more important in the second year. Bacterial community composition was driven by other factors than that of fungi. Fine woody debris harboured more diversified community. Substrate availability, physical properties of plant material and interaction with other saprotrophs seem to be the major drivers of bacterial community development both on litter and deadwood.

Keywords: bacteria, leaf litter, deadwood, decomposition, succession

Abstrakt

Rozklad odumřelé rostlinné biomasy se významnou měrou podílí na koloběhu uhlíku a je tak jedním z klíčových procesů probíhajících v lesích mírného pásu. Zatímco role hub při rozkladu opadu a dřeva byla opakovaně zkoumána, jen málo studií se zabývalo bakteriemi asociovanými s rozkládající se rostlinnou biomasou. Vývoj bakteriálního společenstva asociovaného se stromovým opadem je pravděpodobně způsoben změnami v chemickém složení opadu a dostupností živin. Aktivita hub značně přispívá ke změnám v substrátu a tím je ovlivněno také bakteriální společenstvo. Dostupnost živin se mění v průběhu rozkladu biomasy, na počátku jsou snadno dostupné lehce rozložitelné látky, zatímco později jsou rozkládány odolnější sloučeniny (např. lignin). Bakteriální kolonizace mrtvého dřeva je ovlivněna řadou faktorů, jako například místně specifické klimatické podmínky, druh stromu, objem dřeva. Cílem této práce bylo popsat dynamiku bakteriálního společenstva během dvouletého rozkladu stromového opadu a odumřelého dřeva. V opadovém experimentu bylo bakteriální společenstvo analyzováno v živých, odumírajících a rozkládajících se listech dubu zimního (*Quercus petraea*). Experiment probíhal v přírodní památce Xaverovský háj v České republice. Experiment s odumřelým dřevem byl zaměřen na složení bakteriálního společenstva v počáteční fázi rozkladu větví a kmenů buku lesního (*Fagus sylvatica*) a jedle bělokoré (*Abies alba*). Probíhal v Národním parku Bavorský les v Německu. Složení bakteriálního společenstva bylo určeno sekvenováním genu pro 16S rRNA. Hodnoty enzymových aktivit, houbové a bakteriální biomasy a úbytku suché hmotnosti rostlinného materiálu byly použity k vysvětlení zjištěných rozdílů ve složení bakteriálních společenstev. Zástupci tříd *Alpha-*, *Beta-* a *Gammaproteobacteria* a kmeny *Actinobacteria*, *Bacteroidetes* a *Acidobacteria* převládali v obou experimentech. Byly zaznamenány rody *Sphingomonas*, *Mucilaginibacter*, *Burkholderia* a *Pedobacter*, u nichž byla dříve zjištěna schopnost rozkládat celulózu. Bakterie obývající živé listy byly nahrazeny jinými skupinami krátce po odumření listů. Následný vývoj společenstva prodělal dynamické sukcesní změny způsobené měnící se dostupností živin. Sukcese vedla k diverzifikovanému společenstvu na konci rozkladu stromového opadu. Byly detekovány odlišné strategie prvních kolonizátorů a pozdních specialistů. Průměr rozkládajícího se dřeva ovlivňoval bakteriální komunitu v prvním roce rozkladu, zatímco druh stromu byl určující

v druhém roce. Složení bakteriálního společenstva bylo ovlivňováno jinými faktory než houbové společenstvo. Bakteriální komunita byla více diverzifikována ve větvích než v kmenech. Dostupnost substrátu, fyzikální vlastnosti rostlinného materiálu a interakce s dalšími saprotrofy se zdají být hlavním faktorem určujícím vývoj složení bakteriálního společenstva asociovaného se stromovým opadem a odumřelým dřevem.

Klíčová slova: bakterie, opad, mrtvé dřevo, dekompozice, sukcese

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List of abbreviations

BLASTn – nucleotide Basic Local Alignment Search Tool
bp – base pair(s)
C – carbon
CWD – coarse woody debris (logs with approximately 30 cm in diameter)
ddH_2O – double-distilled H_2O
dsDNA – double-stranded DNA
EB buffer – elution buffer
fastq file – sequence file obtained by Illumina MiSeq sequencing
FWD – fine woody debris (branches with up to 5 cm in diameter)
GH – glycosyl hydrolase
ha – hectare
IUPAC – the International Union of Pure and Applied Chemistry
JGI – Joint Genome Institute
N – nitrogen
NCBI – The National Center for Biotechnology Information
OTU – operational taxonomic unit
PCA – principal component analysis
PCR – polymerase chain reaction
PLFA – phospholipid fatty acids
RDP – Ribosomal Database Project
rpm – revolutions per minute
rRNA – ribosomal RNA
ssDNA – single-stranded DNA
t – ton

1 Introduction

Life on the Earth ultimately depends on biogeochemical cycling, quiet, inconspicuous process which represents continual flow of nutrients and elements between diverse habitats and different sinks. Without this ballanced combination of complex processes life would be significantly reduced or even extincted. It allows the accumulated resources to be recycled, keeps the food pyramid in an equilibrium and enables coexistence of producers and decomposers. This is the case of various life forms, whether talking about chemolithotrophic bacteria in oceanic trenches, CO_2 fixation by temperate forest, grazing of cow on a pasture or predating importance of Blue whale. Although oftenly unconsciously, we are all dependent on this ecosystem functioning.

Ecosystem function is summary of processes typical for given environment and driven by members of all three domains. Processes can be observed at the global scale: biogeochemical cycling, creation of soil structure, nitrogen fixation or plant biomass decomposition. Or at the local scale: substrate utilization by rumen microrganisms, fermentation in bioreactor or methanogenesis in a small pond behind the house. These processes are key for maintenance balanced nutrient and element flow and are crucial for endured life (see Gilbert & Neufeld, 2014).

Some of the key processes responsible for the huge resource turnover are driven by players which were subjected to detailed research only recently and their influence was unveiled not earlier than in the last century. These players are microorganisms, “invisible” and ubiquitous forms of life with rich metabolic apparatus performing often challenging tricks. And one of these challenges is also the decomposition of plant biomass focused in this study.

Together with elevated attention on predicted climate change, emissions of greenhouse gases and carbon cycling during last three decades, more studies have been focused on the role of microorganisms in decomposition of dead plant biomass because of its relevance in the carbon flow. Biomass represents significant stock of this element and mining of carbon from dead plant material (e.g. leaves, tree trunks, branches) actually means its liberation for further processes. Fungi were soon

recognized as strong players in decomposing of biomass due to their enzymatic tools. General mechanisms of fungal degradation as well as factors influencing this process were subjected to research. However, less atention has been given to the bacteria living together with fungi; in dependence to nutrients from either decomposing plant biomass or fungal biomass and struggling with similar challenges as their eucaryote counterparts. Now it is already known that bacteria significantly contribute to the biomass decay through either direct degradation or various symbiotic relationships influencing conditions in the substrate. Nevertheless, elucidation of the extent of bacterial role in the process of decomposition merits further research which will, in the future, shed light on this key part of biogeochemical cycle and will help to confirm or establish micro-ecological theories.

The aim of this study was to broaden knowledge about ecology of bacterial communities associated with decomposing plant biomass regarding community composition, richness and occurence of key taxa under changing conditions. The experiments comprised the analysis of bacterial commnunity development during leaf litter decomposition and during initial phases of decay of deadwood of various quality – the processes with a significant impact in temperate forests.

2 Aims of the study

To describe bacterial community composition and its dynamics during decomposition changes in tree leaves including live and senescent leaves and litter in the early-decay and late-decay phases.

To test whether succession of bacterial taxa follows predictable patterns reflecting chemical and structural changes in litter.

To observe changes in community composition during two years of initial decomposition of deadwood.

To elucidate influence of deadwood species and size on associated bacterial community.

To describe specific bacterial taxa occurring in particular phase of decomposition and thus to allow future assignment of their ecological roles.

To identify similar features of the leaf litter and deadwood decomposition.

3 Literature review

3.1 Temperate forests as important ecosystems

Native forests in temperate zone can be distinguished as deciduous, coniferous, or mixed. Coniferous forests are typical for higher altitudes or for areas intensively managed by forest industry. Here, input of organic matter, in the form of needles, branches or other parts of the plant body, is without strong maximum during autumn as conifers have litterfall distributed over year with less distinct peak. On the contrary, deciduous forests represent original type of vegetation in lower altitudes with characteristic seasonality in organic material input onto forest floor since deciduous trees shed leaves at the end of vegetation season during the autumn. Annual litter amount produced in deciduous forest is estimated to be 3.5 t ha^{-1} (Bray & Gorham, 1964) and therefore represents considerable translocation pathway of photosynthetically fixed carbon from above-ground biomass to topsoil organisms such as saprotrophs.

Temperate forests cover worldwide an area of 5.7 million km^2 (Lindquist et al., 2012). In comparison with other biomes, temperate zone has the lowest total forest cover as a result of transformation to inhabited or agriculture land (Hansen et al., 2010). Moreover, the temperate zone, as well as the boreal or tropic ones, underwent recent deforestration of various extent (Hansen et al., 2010). According to data about tree biomass collected during long-term experiment in temperate deciduous forest, physiology of growing stands is being changed by increased temperature, levels of athmospheric CO_2 and longer growing season. It results in unexpectedly exceeded biomass accumulation and increased growth rate of canopy (McMahon et al., 2010). If this pattern continues, temperate forests will remain one of the key ecosystem, despite deforestration and their occurence in densely populated latitudes.

3.2 Dead plant biomass

Dead plant biomass represents the most abundant source of organic carbon on the Earth. This essential structural element is fixed by the autotrophic process of photosynthesis and serves as the main structural element in plant bodies. Thanks to the recalcitrant structure of cell walls within the biomass, plant matter is extremely resistant. Resistance is provided by biopolymers with tight and inaccessible connections occurring in cell walls. This is important for plants during their lifetime and polymers in their cell walls are crucial for the physical support of their bodies. After plant senescence, dead biomass is being decomposed and this process maintains the flow of nutrients and elements and keeps ecosystem functions. Uncatalyzed degradation of cellulose – the main compound of biomass, would take an incredibly long time. The half-life of its abiotic breakdown was estimated to be higher than 4.5 million years (Wolfenden et al., 1998). Therefore, enzymatic hydrolysis of cellulose is responsible for the majority of carbon turnover via biomass. In the course of evolution, microorganisms have developed rich enzymatic apparatus to cope with recalcitrant substrates present in biomass.

Plant cell wall consists mainly of cellulose, lignin, hemicellulose and pectin. While the primary cell wall is rich in cellulose, pectin can be found in a primary cell wall as well as in a middle lamela (Fig. 1). Moreover, secondary cell wall is typically rigidified by lignin. Higher content of carbon and low concentrations of nitrogen and phosphorus are typical for dead plant matter. As decomposition proceeds, carbon is released in the form of CO_2 by the respiration while most nitrogen remains in the substrate causing decreasing C:N ratio from initial high values. Concentration of lignin in dead plant biomass and high C:N ratio of a substrate can significantly slow down microbial colonization. These properties determine the rate of decomposition and microbial community structure of both litter (Liu et al., 2010; Zhang et al., 2008) and deadwood (Rajala et al., 2011). Furthermore, there is also a negative correlation between C:N ratio and bacterial abundances (Bray et al., 2012).

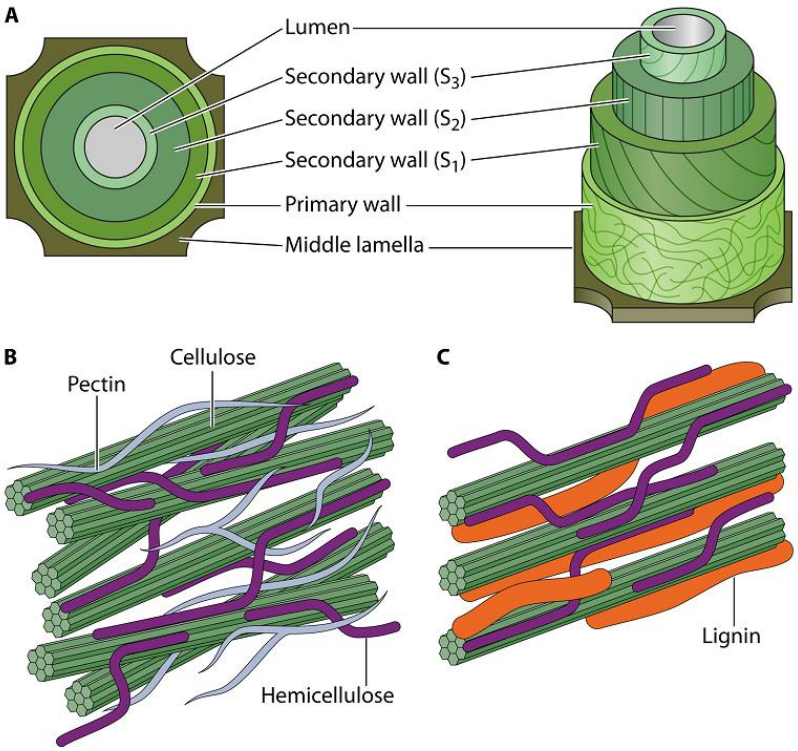


Fig. 1: Parts of plant cell wall and their schematic structure. A – Scheme of cross-sectional view of a plant cell wall structure including layers of middle lamella, primary and secondary wall. B – Main polymers as components of primary cell wall. C – Composition of secondary cell wall rigidified by lignin. (adapted from Rytioja et al., 2014)

3.3 Deconstruction of plant biopolymers

Plant biomass contains several types of different biopolymers, namely cellulose, hemicellulose, lignin, starch, pectins, tannins and others. These compounds are of different chemical structure and they can be found as a part of the cells of plant tissue or as the part of the cell wall. Biopolymers serve either as structual components of plant tissue providing toughness and rigidity to the plant body or as an energy storage in the case of starch.

For saprotrophic microorganisms, decomposition of structurally complex dead plant biomass can be imagined as a dilemma because of the rigid plant biopolymers and difficulties with their degradation on one side and energetic potential caused by high carbon content on the other side. But microorganisms cope with recalcitrance of plant biomass successfully because, throughout the evolution, saprotrophs developed strong enzymatic machinery enabling successful decomposition of plant biomass and

gaining nutrients from it. From the evolutionary perspective, microrganisms have had enough time for acqusition of diverse metabolic pathways helping them to utilize different substrates effectively. For example, 66.7% from 8 133 sequenced bacterial genomes showed potential to degrade either cellulose, xylan (part of the hemicellulose) or chitin based on searching for glycosyl hydrolase genes (Berlemont & Martiny, 2015). Polysaccharides and glycosyl hydrolases are discussed in the text below.

It is not only the complex chemical structure what limits carbon uptake from dead plant biomass. Carbon accessibility is under strong influence of environmental factors (Schimel & Schaeffer, 2012). In the case of forest topsoil these can be climatic conditions, chemical properties of each specific site, differentiation of micro-niches causing heterogeneity of soil (Vos et al., 2013), nutrient aggregation and binding of leached soil organic matter to particles in lower parts of soil causing physical unavailability of carbon in the topsoil (Dungait et al., 2012; Schimel & Schaeffer, 2012).

As was mentioned above, the volume of globally produced plant material to be decomposed is huge and therefore processes connected with saprotrophic degradation significantly contribute to the cycles of carbon and other nutrients. Beside decomposition performed by invertebrates and vertebrates, there is microbial decomposition, occurring at high rates, which is performed by fungi and bacteria. Their relative contribution to process of decomposition is also discussed in the text below. The following section is devoted to the description of key enzymes with the focus on bacterial ones. Their production and activity occur in the habitats of soil, leaf litter and decomposing wood – sites where bacteria strive to degrade biopolymers.

3.3.1 Cellulolytic enzymes – bacterial perspective

Cellulose is the globally ubiquitous organic compound. It occurs mostly as the component of plant biomass where it is the main component of the cell wall. Its function is to protect cells and serve as a scaffold for maintaining their shape. Composition of a cell wall differs according to the plant species but structure of cellulose remains identical. Cellulose is a polymer of D-glucose units connected by

β-1-4 glycosidic bonds. Oligomers of more than 6-7 monomers are insoluble (Warren, 1996). Cellulose fibrils are up to thousands of units long and are essential structures for the cell wall composition. Cellulose polymer contains more labile amorphous regions and more recalcitrant crystalline regions. The latter are inaccessible for enzymatic cleavage of glycosidic bonds. To recalcitrant nature of cellulose contributes also its association with other biopolymers, namely lignin and hemicellulose (Fig. 1). Separate section in this thesis is devoted to lignin. Hemicellulose is heteropolymer of D-glucose and other sugar monomers such as xylose, mannose, galactose. Its degradation is performed by xylanases.

Cellulose can be utilized through several ways (reviewed by Wilson, 2011). In the anaerobic conditions, cellulose degradation is carried out by bacteria possessing cellulosomes (Bayer et al., 2004; Doi & Kosugi, 2004). These structures on the surface of cells are assembled from different enzymes which operate synergistically (Lynd et al., 2002). Substrate is degraded effectively step by step by multiple enzymes present in the cellulosome. Thanks to the attachment of cellulosomes to bacterial cell surfaces they do not diffuse to the substrate, products of degradation thus remain in the proximity of the cell and are accessible preferentially to the producer. Cellulosome strategy was described for the genera *Clostridium*, *Ruminococcus*, *Bacteroides* and *Acetivibrio* (reviewed by Schwarz, 2001).

Aerobic bacteria mainly targeted in this thesis produce freely diffusible enzymes (as do also fungi). These are secreted outside the cell and liberated products are accessible to all potential users in the vicinity. Such a strategy can result in the so called “cheating behaviour” (Velicer, 2003) where microorganisms profit from the enzyme production of their neighbor saving their own resources. In this relationship, persistence of producer microorganisms and success of cheaters depends on the costs of enzyme production, rate of enzyme diffusion (Allison, 2005) and quality of the resource itself (modelled in the Allison, 2012). Besides cheating, efficiency of cellulose utilization can be affected by the morphology of bacterial cells. Several members of the phylum *Actinobacteria* exhibit mycelial growth and it allows them to pervade substrate and better exploit nutrients. This way, *Actinobacteria* can resemble growth of fungi for which filamentous growth is common.

Three types of enzymes (glycosyl hydrolases, GH) can be distinguished according to the mode of action on cellulose: endocellulase (endoglucanase, EC 3.2.1.4), exocellulase (cellobiohydrolase, EC 3.2.1.91 acting on non-reducing, EC 3.2.1.176 acting on reducing end) and β-glucosidase (EC 3.2.1.21)(Lynd et al., 2002). All of them posses specificity for cleavage of β-1-4 glycosidic bond. Endocellulase acts on random position of amorphous regions and cleaves cellulose fibril producing reducing and non-reducing ends of the chain. Both ends can be targeted by exocellulases. They act in a unidirectional mode producing the disaccharide cellobiose. Molecules of cellobiose have some interesting properties. Cellobiose is a repressor of cellulosome synthesis (Zhang & Lynd, 2005) and its high concentration can inhibit cellulases (Holtzapple et al., 1990). Lastly, β-glucosidase cleaves glycosidic bond in cellobiose producing two monomers of D-glucose (Warren, 1996) which are than metabolized.

The key part of each glycosyl hydrolase is its catalytic domain, sometimes linked by an extended linker to one or more carbohydrate binding modules. Glycosyl hydrolases have been sorted to the families according to their structural and reaction similarities. For maintaining of systematics within different glycosyl hydrolase families the CAZy database (Carbohydrate-Active enZYmes database, http://www.cazy.org/) was set up (Cantarel et al., 2009). In 2015, there were 133 GH families and 220 910 classified domains. Bacterial cellulolytic enzymes are members mainly of the families GH1, GH3, GH5, GH6, GH8, GH9, GH12, GH44, GH45 and GH48, while xylanases responsible for hemicellulose degradation are known from the families GH10, GH11 and GH30 (Berlemont & Martiny, 2013, 2015). Searching for GH genes in 8 133 sequenced bacterial genomes unveiled potential for breakdown of cellulose and hemicellulose in 40.8 and 24.6% of these genomes, respectively. 76.9% of potential cellulose degraders exhibited putative ability to degrade chitin (Berlemont & Martiny, 2015).

In the study of Berlemont & Martiny (2013) 5 123 sequenced bacterial genomes were subjected to search for genes encoding potential enzymes involved in cellulose degradation. As a results 21 985 genes were compared with CAZy database and these genes were affilliated to the GH families. Enzymes from families GH1 and GH3 were the most abundant in examined bacterial genomes. They appeared to be mostly β-glucosidases. Enzymes from GH5 family almost exclusively showed endocellulase

activity. Enzymes from GH6 and GH9 families were endocellulases and also exocellulases. GH8 family members were endocellulases. GH12 were almost completely endocellulases. Enzymes from GH44 and GH45 were endocellulases and exocellulases, meanwhile GH48 family contained almost exclusively exocellulases. Last three families can be found as a part of cellulosomes. At least one gene belonging to either GH1 or GH3 (the most abundant families in the study) was found in 79% of genomes and in nearly all the bacterial phyla (Berlemont & Martiny, 2013). GH5 was the family which encompassed the most abundant putative genes for endo- and exocellulases. These genes were detected in the genomes of *Actinobacteria*, *Bacteroidetes/Chlorobi* group, *Chloroflexi*, *Fibrobacteres*, *Acidobacteria*, *Firmicutes*, *Proteobacteria* and *Thermotogae*. It suggests that these phyla are capable of degrading cellulose fibrils. While 24% of the genomes contained genes for hypothetical cellulases and also β-glucosidases, 56% of the genomes possessed genes only for β-glucosidases. Bacteria with the latter mentioned genomes can be regarded as opportunists or cheaters utilizing products of enzymatic cleavage performed by cellulase producers. This strategy is probably widespread and therefore can be considered as limited indicator of cellulolytic potential of bacterial community. Noteworthy, decomposition of plant biomass is so dependent on these relatively minor and specialised groups with cellulase genes. Discussed *in sillico* approach of Berlemont & Martiny, (2013) can (despite the plausible skewed dataset towards pathogens and identification of genes on the putative basis) provide us the preview of phylogenetic distribution of functional strategies among bacterial representatives with known genome.

3.3.2 Lignin-modifying enzymes

Lignin is aromatic polymer of monolignols paracoumaryl alcohol, coniferyl alcohol and sinapyl alcohol found in the lignin in the form of phenylpropanoids (Wong, 2008). The ratio of the phenylpropanoids varies at the genus level in both gymnosperms and angiosperms. Polymerization of lignin is done by treatment of reactive radicals. Chains of lignin are interlinked by carbon (C_α-C_β) and ether bonds. Resulting complex structure is heterogenous and it serves for increased recalcitrance of plant tissues. Lignin also protects cellulose from enzymatic degradation by reducing

its accessibility for enzymes. During decomposition, lignin remains in the substrate as the last compound of the plant biomass (Šnajdr et al., 2011). In average 20% of carbon fixed by photosynthesis is incorporated into lignin (Ruiz-Dueñas & Martínez, 2009) and so production of lignin by plants is worldwide significant. Together with cellulose it plays relevant role in the biogeochemical cycle of carbon.

Degradation of lignin is mediated by chemical reactions of ligninolytic enzymes, radicals and mediators. Direct contact of active site of the enzyme and lignin is thus not necessary. Two groups of lignin-degrading enzymes can be distinguished. (i) peroxidases such as lignin peroxidase, Mn-peroxidase and versatile peroxidase which are exclusively produced by fungi and (ii) phenoloxidases such as laccases produced by both fungi and bacteria.

Laccases (EC 1.10.3.2) are multi-copper oxidoreductases with broad substrate specificity. Reaction centre of these enzymes comprises at least 4 copper atoms held in conserved regions. During substrate oxidation, molecule of oxygen serves as an electron acceptor and is reduced to water. Reactive radicals are produced by oxidation and are responsible for different possible reaction mechanisms: polymerization, degradation of polymers or degradation of phenolic compounds (Claus, 2003, 2004). Moreover, laccase activity can produce mediators – oxidized small molecular-mass compounds, responsible for oxidation of various phenolic substrates (e.g. lignin). Laccases have been studied mainly in fungi (Baldrian, 2006), however, their occurrence in the domain *Bacteria* is also well described (Alexandre & Zhulin, 2000; Sharma et al., 2007).

Laccases catalyze variety of (sometimes contradictory) processes in organisms. Plant laccases participate in lignin synthesis (Bao et al., 1993; Ranocha et al., 2002). Conversely, for white rot members of *Basidiomycota* laccases enable modification of lignin structure which may helps with its following degradation (Thurston, 1994). In the field of bacterial laccases, research has been focused for example on notoriously mentioned laccase CotA in a coat of *B. subtilis* spore (Hullo et al., 2001) and on resistant laccases with possible use in industry (Miyazaki, 2005; Niladevi et al., 2008).

Ecological role and contribution of laccases to the process of biomass decomposition still need deeper studies. Laccase-related questions can be answered with the help of growing amount of available data. These can be represented by the

database of sequenced bacterial genomes in NCBI and JGI which grows very rapidly due to advanced molecular techniques (e.g. Zhang et al., 2006) allowing sequencing and phylogenetic affilliation of even uncultivated bacterial taxa from diverse environments with potentially interesting and unprecedented metabolic abilities (Rinke et al., 2013). Deposited sequences can be used for data mining with intention to reveal genes for laccase-like enzymes. It can help with indentification of bacterial taxa with potential for lignin modification. This approach was used by Ausec et al., (2011) for complete or draft genomes and 4 metagenomic datasets. These data were subjected to search for laccase genes using the identification based on the Hidden Markov Models. Results provided 1 240 putative genes for these enzymes from 36% of studied organisms while database covered 2 211 genomes. Interestingly, 76% of putative proteins posses signal peptides suggesting their localization outside the cell. Although identification of laccase-like genes does not mean that bacteria has laccase activity and not all lacasses account for lignin modification, this study reveals how much is predisposition of laccase transcription widespread in bacteria.

In conclusion, database for laccase classification and collection of laccase-related molecular data was established under the name Laccase Engineering Database (LccED) (Sirim et al., 2011). Lignin-modifying enzymes are also listed within the CAZy database as auxilliary activity molecules.

3.3.3 Other biopolymers and enzymes

Besides cellulose, hemicellulose and lignin, microorganisms can also degrade other biopolymers in decomposing biomass. Pectin represents possible source of carbon although its importance is not as high as in the case of cellulose or hemicelluloses. Pectin is a homopolymer composed of units of D-galactouronic acid linked by α-1-4 bonds. Similarly as for cellulases, pectinases can be divided according to their site of acting to endopectinases (EC 3.2.1.15) and exopectinases (EC 3.2.1.67) (Favela-Torres et al., 2006). Pectin-degrading enzymes were found for example in the group of actinomycetes (Brühlmann et al., 1994) and more specifically in the genus *Streptomyces* (Beg et al., 2000).

Chitin is often neglected as substrate available in the habitat of decomposing plant material. Chitin is composed of β-1-4-linked monomers of *N*-acetyl-D-glucosamine arranged into micro-fibrils. It varies in the level of deacetylation. Completely deacetyled form of polymer is known as chitosan. Further deamination of chitosan can provide cellulose-like forms of polymers. Chitin serves as a structural polymer of the fungal body and as fungal biomass on decomposing substrate is typically high, chitin may represents a relevant source of energy. Its global production is estimated at approximately 10^{10}–10^{11} tons $year^{-1}$ (reviewed by Beier & Bertilsson, 2013). Despite this enormous production, it is not accumulated in the environment, which denotes its efficient turnover. Chitin degradation is mediated by chitin-degrading enzymes – chitinases (EC 3.2.1.14, distinguished as endochitinases and exochitinases) and chitosanases (EC 3.2.1.132). The ability to degrade chitosan was also described for lysozyme (EC 3.2.1.17) and some cellulases (GH5, GH7 and mainly GH8)(Xia et al., 2008). Besides other organisms, also certain bacteria posses chitin-degrading enzymes. Chitinases were detected in glycosyl hydrolase families GH18 and GH19 (Berlemont & Martiny, 2015). Potential for chitin degradation was detected in 53.1% of sequenced bacterial genomes and 74.8% of these putative degraders showed predispositions for degradation of plant polysaccharides (Berlemont & Martiny, 2015). The same study identified chitinases in taxa *Cytophaga*, *Flavobacterium*, *Actinomyces* and groups of *Proteobacteria* and *Firmicutes*. Further, Vaaje-Kolstad et al., (2013) identified chitinolytic activity in *Serratia marcescens*.

Interestingly, not all members of the bacterial community that utilize chitin harbour genes for its degradation. Thus, the number of chitin-degraders can be lower than the number of chitin-consumers due to cross-feeding similarly as in the case of cellulose (reviewed by Beier & Bertilsson, 2013). Phylogeneticall composition of chitin-degrading bacterial community in soil microcosms has been shown to be changed by chitin amendment. This amendment simultaneously caused, at community level, increase in copy numbers of *chiA* gene which is involved in degradation of chitin (Kielak et al., 2013). Question for further research is, if there is a link between chitin content and bacterial community composition also in the habitat of decomposing plant biomass. Here fungal biomass serves as a chitin supply, either in the form of live fungi or dead bodies which remain in the substrate and represent possible resource for

bacteria. Relationships between mycophagous bacteria and their fungal counterparts where chitin degradation may be involved is focused later in this thesis (section 3.6).

3.4 Bacterial community in soil, litter and deadwood

3.4.1. Soil as a source of bacterial diversity

Soil is considered to be the source of bacteria identified within the decomposing biomass and even phyllosphere (Copeland et al., 2015; Vorholt, 2012). This section describes soil due to its similar properties with leaf litter and deadwood habitat. Similar drivers of the composition may influence bacterial communities in these interconnected environments. Soil exhibits high species richness and also abundances of bacteria. Counts of bacterial taxa at the level of species are roughly estimated at tens of thousands for one gram of soil (Curtis et al., 2002; Roesch et al., 2007; Torsvik et al., 1990). Bacterial taxa can be identified based on the sequence of 16S rRNA gene (Fig. 2). The same amount of soil can host up to 1 billion of bacterial cells until free niches are saturated (Roesch et al., 2007). Such a high numbers of soilborne bacteria can be explained by big amount of relatively easily accessible nutrients in this habitat. Studies also emphasize the heterogenous character of soil environment (Raynaud & Nunan, 2014; Vos et al., 2013) allowing microorganisms to create diverse communities as well as to build rich ecological interactions with their neighbors.

Although according to some indications number of possible interactions with surrounding partners is limited regardless of the overall community richness of concrete site (Raynaud & Nunan, 2014). From the perspective of individual bacterial cell soil is complex habitat consisted of particles that create three-dimensional structure filled by pores of water and air. For unicellular bacteria it is demanding to span these cavities and so bacterial motility in soil is limited. Factors of moisture and spatial heterogeneity (i.e. amount of space between aggregates of particles, clay, silt, sand etc.) seem to be among the most important for spreading of bacteria and so maintaining diversity (Vos et al., 2013).

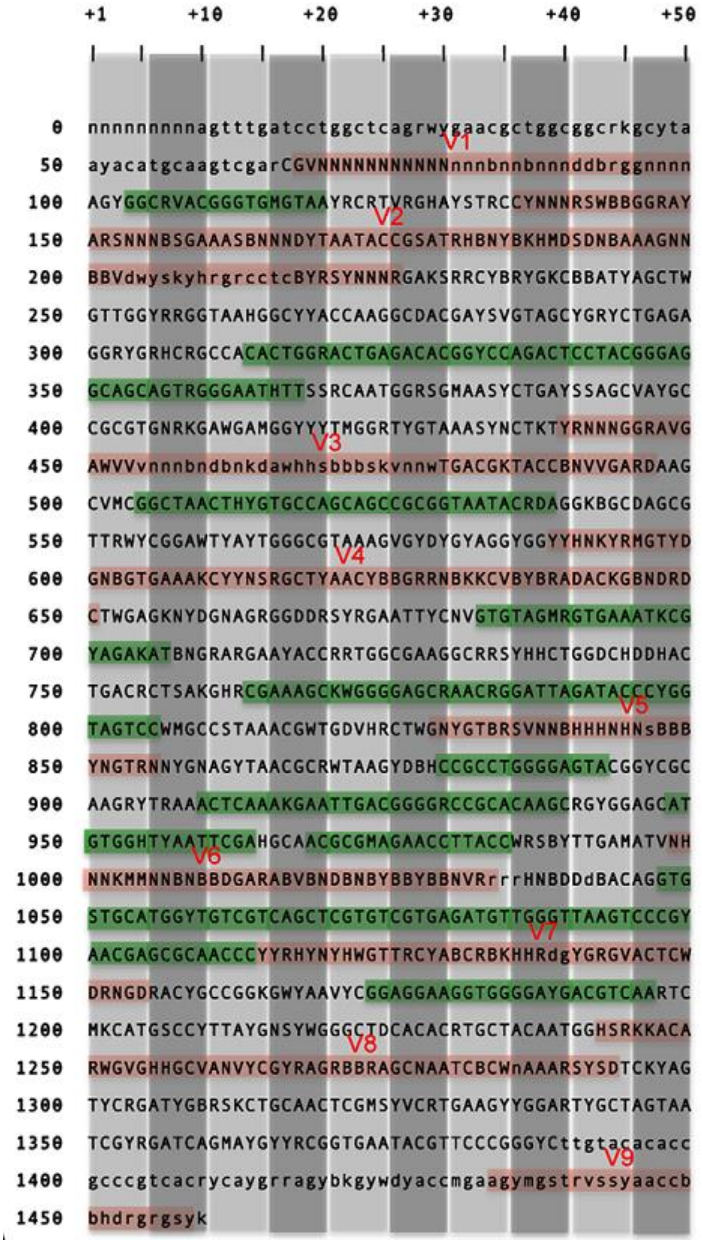


Fig. 2: Consensus sequence of the 16S rRNA gene as extracted from 41 109 soil-bacterial entries in RDP database with 90% conservation cut-off value. Letters for nucleotide identification are in accordance with IUPAC system. Red boxes stand for hypervariable regions V1-V9 and green boxes stand for possible primer designing sites. Lower-case letters indicate position where gaps constituted more than 10% in the alignment. (adapted from Vasileiadis et al., 2012)

Not only patchiness of the soil determines composition of community by restriction of bacterial movement and number of interactions. Community composition is also determined by a range of other environmental variables. Notably, diversity and composition is under the control of pH (Lauber et al., 2009; Rousk et al., 2010; Urbanová et al., 2015) which seems to be reliable predictor of community

composition at larger spatial scales (Lauber et al., 2009). Season and annual cycle also contribute to shaping bacterial community in soil (Cruz-Martínez et al., 2009; López-Mondéjar et al., 2015). Moreover, community is under direct influence of vegetation type (Urbanová et al., 2015) and soil type (Griffiths et al., 2011).

Forest floor in temperate forests is heterogenous with distinguishable layers of litter and soil which differ in structure, nutrient content and also community composition (Šťursová et al., 2012). Higher fungal and bacterial biomass content was revealed in litter than in the soil (Šnajdr et al., 2008; Šťursová et al., 2012) as well as higher evenness and richness of bacterial community in litter layer (Urbanová et al., 2015). Higher amount of carbon derived from cellulose was accumulated in bacterial biomass than in fungi indicating preferential utilization of litter by bacteria. In soil, bacterial:fungal biomass ratio was higher than in litter and cellulose was more utilized by fungi (Šťursová et al., 2012). Cellulolytic bacteria represented only part of the total community and the composition of their communities changed along the soil profile. The genera *Herminiimonas* and *Mucilaginibacter* followed by *Pedobacter* and *Streptadiciphilus* were detected among the cellulolytic taxa as the most abundant in both litter and soil of an acidic coniferous forest. In litter, community utilizing cellulose was also rich in *Cytophaga* and *Asticcacaulis*, while *Alkanindiges* and *Collimonas* were abundant in soil (Šťursová et al., 2012). Generally, bacteria in soil belonged to the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* which was confirmed also by López-Mondéjar et al., (2015).

For bacterial communities focused in this thesis, soil communities can serve as the source for colonization of dead plant biomass. Majority of mentioned factors and principles plays similar role in determining the composition of the plant biomass-associated community. Its properties are described herein.

3.4.2. Leaf litter species and chemistry

The process of litter decomposition was subject of comprehensive research since the second half of the 20th century (Prescott, 2010). Nevertheless, there are still gaps in the knowledge regarding microbial role in litter biomass turnover. One of such gap is composition of bacterial community during transition from live to senescent leaves

targeted in this study. When considering community composition of bacteria on decomposing leaf litter it is important to take into the account the quality of leaf biomass. It differs according to tree species under which the decomposition takes place. Leaf species and differences in chemistry of biomass have significant effect on bacterial community composition (Aneja et al., 2006; Urbanová et al., 2015; Wardle et al., 2006). For example, difference in the composition of the classes of the phylum *Proteobacteria* was described for beech litter (dominance of *Gammaproteobacteria*) and spruce litter (dominance of *Alpha-, Beta-, Gammaproteobacteria*)(Aneja et al., 2006). Experiment focused on composition of litter- and soil-associated microbial community under six planted genera of trees and one site with spontaneous succession showed effect of tree species as significant driver of microbial community composition in both litter and soil (Urbanová et al., 2015). Tree species effect on bacterial community is likely mediated by leaf litter and specific pH. Moreover, bacteria seem to be generalists in comparison with fungi which were described as more tree specific (Urbanová et al., 2015).

Other variables such as local climate (Kubartová et al., 2007), and mixing of substrate play also important roles although the latter variable is less significant in comparison with leaf litter identity (Wardle et al., 2006). This was shown also in the study comparing bacterial communities from forests of various tree composition where bacterial commnunities differed in dominant bacterial taxa (Hackl et al., 2004). The complex relationship between leaf species, its chemistry, available nutrients and community composition is therefore evident. Moreover, effect of litter chemistry and availability of nutrients (more than *total* content of nutrients) determines the decomposition rate (Urbanová et al., 2014). Litter-species effect likely dominates at initial phase of decomposition while community composition is more important factor for the decomposition rate in the later phases (Bray et al., 2012).

Dominant trees may have influence on community structure and activity of extracellular enzymes via leaf litter species (Šnajdr et al., 2008). However, it was shown, that tree species do not affect species richness of microbial community (Kubartová et al., 2007). Further, experiment with chronosequence of three post-mining sites showed dependence of litter-associated microbial community on decomposition stage as more important than on litter source (Urbanová et al., 2014).

3.4.3. Successional changes in community throughout litter decomposition

Starting from the very beginning of the decomposition, phyllosphere bacterial community should not be omitted as it represents major portion of biota associated with live leaves (reviewed by Vorholt, 2012). Specific bacteria form consortia surrounded by extracellular matrix on the relatively extreme surfaces of leaves or in their internal parts. On the surface they must deal with nutrient limitation, UV radiation, changing humidity and temperature and are influenced by biotic factors such as leaf age or competition with other microorganisms. Nonetheless densities of bacterial cells associated with leaves are estimated to 10^8 cells cm^{-2}, more than fungi or *Archea* (Meyer & Leveau, 2011). Bacterial community possibly affects growth and health of the host plant (Innerebner et al., 2011) and therefore can be considered as a kind of plant probiosis. Proteogenomics of phyllosphere community of three plant species revealed interesting metabolic abilities of two dominant genera – *Sphingomonas* and *Methylobacterium* (Delmotte et al., 2009). *Sphingomonas* highly expressed TonB-dependent receptors for transport of nutrients from its surroundings suggesting broad substrate preferences. *Methylobacterium* as methylotrophic organism gains methanol directly from the plant where it is produced as a side product of plant-cell wall metabolism. Thanks to these distinct life strategies both genera can show co-occurrence (Delmotte et al., 2009). Phyllosphere of grasses may also harbour bacteria capable of ammonia oxidation which contributes to the production of N_2O from pastures (Bowatte et al., 2014) – the ability previously attributed exclusively to soil microorganisms.

Interestingly, bacterial phyllosphere community seems to be specific for dissimilar tree species as shown in a study comparing 10 species (Redford et al., 2010). According to the results, phylogeny composition of bacteria corresponded to phylogeny of the trees. Notably, this was observed even on larger spatial level, when geographic distance had little influence on the variability of phyllosphere community of *Pinus ponderosa* (Redford et al., 2010). Similarly minor geographical role (although on smaller scale) was observed also in (Copeland et al., 2015). In this recent study

comparing annual plants, the plant species effect was not important, possibly due to the influence of soil microbiota.

Changes in community composition in phyllosphere during the course of vegetation season have revealed highly variable community which showed a non-random seasonal pattern (Redford & Fierer, 2009) and probably followed the changes in leaf chemistry. Community in this case could be divided into early, middle and late. The most significant changes were observed for the bacterial genus *Pantoea* from the family *Enterobacteriaceae*. Although decreasing species richness of the phyllosphere towards leaf-adapted community was observed during vegetation season of annual plants (Copeland et al., 2015), variability in microbial diversity of tree phyllosphere did not follow any evident pattern (Redford & Fierer, 2009).

Considering succession of bacteria on substrate, one can distinguish different life strategies as were established in macro-ecology. *r*-selected species are the first colonizers utilizing labile substrates. On the contrary *K*-selected species are specialized for exploitation of recalcitrant compounds such as lignin, they are more effective, with expansion in latter stages of decay (Dilly et al., 2001). Similar pattern was predicted in theoretical model of Moorhead & Sinsabaugh (2006) where decomposing functional groups (referred as guilds) were called “opportunists”, “decomposer specialists” and “miners”. These groups differ according to rate of growth and substrate preferences from group with quick growth and affinity for labile soluble compounds to group with slow growth and specialization for lignin degradation. The alternation of representatives of different life strategies during litter decomposition was confirmed in the microcosm experiment (Rinkes et al., 2011). Data of enzyme activities from the *in situ* experiment suggest similar course of strategies focused first on labile substrates and later on more recalcitrant ones (Šnajdr et al., 2011). Leaf litter decomposition is, moreover, characterised by influence of season and annualy repeating input of senescent leaves. In this way, mentioned strategies are closed in cycle where occurrence of particular guilds of microorganisms can be only ephemeral. Seasonality factor influencing communities in leaf litter was assesed in the case of fungi (Voříšková et al., 2014) and also bacteria (López-Mondéjar et al., 2015).

Among others, decomposition of leaf litter was previously studied by Šnajdr et al., (2011) and the material from their experiment was used in this thesis (Fig. 3). The

study comprised chemical analysis of litter, measurement of enzyme activities and quantification of microbial biomass. It revealed that the process of *Quercus petraea* litter decomposition could be divided into the three stages. The first stage began after leaf senescence and lasted until month 4. Exocellulases, β-glucosidases and β-xylosidases were the most active enzymes. Despite snow coverage of the site during this initial phase, biomass loss was significant and represented 16.4%. Hemicellulose was most rapidly lost. Between months 4 and 12, activities of endocellulose and endoxylanase peaked. Cellulose was the most rapidly degraded source of carbon and total biomass loss was 31.8%. In the last phase, enzymes acting on recalcitrant substrates were the most active, typically laccases acting on lignin. After two years of decomposition, the total loss of litter mass was 67.9% (Šnajdr et al., 2011). It is clear that substrates are utilized at dissimilar rates and that decay begins from the most labile compounds and continues towards more recalcitrant ones. Resolution into the three stages of decomposition (Šnajdr et al., 2011) thus matches with concept of three guilds of microorganisms predicted by (Moorhead & Sinsabaugh, 2006).

Analyses of dynamics of microbial biomass showed predominance of fungal endophytic biomass in live leaves collected two months before senescence (Šnajdr et al., 2011). These fungal representatives likely persisted during the senescence and constituted dominant part of fungal community in the initial phase of decomposition (Voříšková & Baldrian, 2013) by out-competing other microbes such as bacteria (Mille-Lindblom et al., 2006). Very dynamic alteration of dominant fungal taxa was observed when the development of the fungal community composition was studied in this initial phase suggesting strong competition for labile substrates (Voříšková & Baldrian, 2013). It offers question if bacterial community undergoes the same pattern. As decomposition continued, bacterial biomass increased and the fungal:bacterial ratio decreased from initial high values. It seems that bacteria were growing at higher rates in later phases which was further observed in another experiments with decomposing leaf litter (Purahong et al., 2014, 2015). Urbanová et al., (2014) showed similar decrease of fungal:bacterial ratio during 2 years of litter decomposition which was, however, caused by decrease in fungal biomass.

1 **2**

Fig. 3: Leaf litter from litterbags after 6 moths (picture 1) and 18 months (picture 2).

Bacteria associated with decomposing leaf litter were phylogenetically assigned to the following phyla: *Actinobacteria*, *Acidobacteria*, *Bacteroidetes* and *Proteobacteria* (Baldrian et al., 2012; Štursová et al., 2012; Urbanová et al., 2015). Recently published studies provide further insight into bacterial community on leaf litter at the sufficiently fine level of taxonomy. Effect of seasonality and following change in bacterial community composition in litter and soil was investigated at the study site of the leaf litter experiment in this thesis (López-Mondéjar et al., 2015). The most abundant bacterial phyla in litter and soil were similar as those listed above. In the litter horizon, *Bacteroidetes* and *Proteobacteria* (*Alpha-, Beta- and Gamma-*) were the most abundant phyla. Abundance of *Acidobacteria* was increasing with the soil depth. *Mucilaginibacter*, *Pedobacter*, *Ferruginibacter*, *Bradyrhizobium*, *Rhizomicrobium*, *Rhodanobacter*, *Burkholderia*, *Rudaea*, *Luteibacter*, *Sphingobacteria*, *Pseudomonas* and *Novosphingobium* were the most abundant bacterial genera. From the phylum *Acidobacteria*, genera *Granulicella* and *Edaphobacter* were abundant in the litter and organic soil horizon. Bacterial community was most influenced by the horizon. Moreover, 58% of OTUs in the litter horizon also significantly differed in their relative abundances among seasons: samples from spring and summer significantly differed from those from autumn and winter (López-Mondéjar et al., 2015). According to these results, bacterial community in a deciduous forest undergoes pronounced seasonal changes in composition which are horizon-specific. While change in lower soil horizon may be caused by fluctuating amount of root exudates throughout the year, bacterial community composition in litter horizon may undergoes shift due to change in quality of decaying litter (López-Mondéjar et al., 2015), which was,

however, not yet confirmed. Strong influence of bacterial community composition by seasonal and annual variation seems to be even more pronounced (explained 14-39%) than influence by altered abiotic factors such as reduced precipitation or nitrogen addition (explained 2-6%)(Matulich et al., 2015). Bacterial communities in litter of annual plants in the cited study were dominated by few abundant bacterial OTUs which were assigned as *Duganella*, *Curtobacterium*, *Frigoribacterium* and *Kineococcus*. These OTUs together represented around 40% of all sequences.

The dynamics of composition of bacterial community during leaf litter decomposition, however, still merits detailed studies with finer resolution of individual bacterial taxa.

3.4.4. Lignin degradation

Bacterial lignin degradation has been studied since the middle of the 20th century (reviewed by Clausen, 1996). Energy gain from cleavage of lignin seems to be minor and insufficient to support bacterial growth. Therefore, rather than for lignin degradation, laccase activity serves for degradation of lignin-solubilization byproducts or potentially inhibiting substances [such as mono- and oligophenols or polychlorinated biphenyls in polluted sites – ability known for *Rhodococcus jostii* (Ahmad et al., 2010)] which may arise in the bacterial surroundings due to metabolism of other bacteria or fungi in the substrate (Bugg et al., 2011). However, this is not the case of submerged or generally anoxic substrates, where bacteria may play major role in biomass decomposition. Unfavourable growth conditions for fungi, loss of advantage of hyphal form (in submerged biomass) and benefit of direct cleavage by cellulosome in anoxic environments allows outcompeting of fungi by unicellular bacteria (DeAngelis et al., 2011).

Bugg et al., (2011) mentioned the groups actinomycetes, *Alphaproteobacteria* and *Gammaproteobacteria* as lignin-modifying possesing genes for breakdown of lignin. Genes for laccase-like enzymes can serve as predictors of lignin-modifying potential (Ausec et al., 2011). However, lignin breakdown has limited rates as was shown for actinomycetes (Pasti et al., 1990; Větrovský et al., 2014). Study of Větrovský et al., (2014) recorded mineralisation of up to 1.1% of lignin by certain strains of

actinomycetes which reveals only minor cometabolic respiration of this polymer together with cellulose and hemicellulose. Bacterial lignin-modifying genera are further comprehensively reviewed in the study of Tian et al., (2014). For example, *Sphingomonas paucimobilis* SYK-6 can use PCBs, herbicides and lignin-derivates as a sole carbon source and therefore became model organism for bacterial catabolism of aromatic substrates. The genus *Pseudomonas* was among the first characterized for lignin degradation. Some filamentous members of the genus *Streptomyces* are known for their lignin degrading activity as well as plant pathogenicity. Another lignin-degrading bacterium is *Streptomyces viridosporus* T7A. *Rhodococcus jostii* RHA1 has fully sequenced genome which is one of the largest known in the domain *Bacteria* (McLeod et al., 2006). It comprises many genes involved in predicted pathways for degradation of aromatic compounds (including lignin and PCBs). *Thermobifida fusca*, thermophilic actinomycete, features thermo-tolerant laccases and cellulases. This is just short summary of lignin-modifying genera. Detailed information can be found in the review (Tian et al., 2014).

3.4.5. Deadwood decomposition

Regarding lignin and deadwood degradation, it is assumed that main players in lignin and lignocellulose degradation in terrestrial ecosystems are fungi from the group of white-rot *Basidiomycota* (Baldrian & Valášková, 2008). Fungi have potential to significantly change conditions in substrate, exclusively produce efficient ligninolytic peroxidases (Kirby, 2006; Møller et al., 1999; Schneider et al., 2010) and seem to be more efficient during deadwood decomposition in comparison with bacteria. Moreover, mycelial growth form – key feature of fungi, contributes to thorough substrate exploitation by physical penetration of tissues and enables nutrient translocation (Boer et al., 2005). Nevertheless, bacterial deterioration of deadwood can not be excluded. Owing to the structural changes in material caused by enzymes bacteria were divided into tunneling and erosion group (Clausen, 1996). The first group invade outer 1 cm of wood and cause penetration of S_3 layer of the cell wall while the erosion group cause formations such as channels and depressions. Altered

permeability of wood cells may assist the infiltration of large fungal enzymes (Clausen, 1996).

Deadwood represents significant carbon stock in ecosystems and its decomposition is of special interest of foresters, ecologists and climatologists. Data about deadwood decay are important for the quantification of carbon flow from plants to soil and to the atmosphere. Therefore, they can serve as a tool for development of ecological models estimating carbon stocks and turnover, not to mention the commercial aspect by which forests are surely viewed. Factors which influence decomposition of deadwood were subjected in many studies as well as the role of saprotrophic fungi (Rajala et al., 2011), size of wood during the course of decomposition (Fraver et al., 2013) and soil chemistry changes in the surrounding of a log (Bade et al., 2015).

Trees represent the link between the atmosphere and the soil in the flow of various substances. In the case of carbon, this flow is mediated by photosynthesis, production of exudates via roots into the soil, interception of leaf litter and decomposition of dead plant biomass. Different environments are so connected via this flow. After the death of a tree the decomposition is initiated and changes in chemistry start to appear. Wood, bark and stemflow water show changes in chemistry in the means of altered concentrations of elements such as P, Mg, Al, Mn, Zn, K (Bade et al., 2015). In contrast to the wood decomposing on soil surface, the surrounding of standing deadwood shows only slight changes in chemistry caused by deadwood decomposition. In the study of Bade et al., (2015) changes were represented by increased base cation and Mn concentration downhill from the standing deadwood. Slow rate of nutrient release into the soil can be partly explained by conditions of the sampling site in the mentioned study (slow wood decomposition and generally low nutrient availability in Harz National Park) but also by general phenomenon that decomposing logs are densely covered by bryophytes and vascular plants. Also the surrounding of the log provide advantageous conditions for fast growth of other trees. Log's coverage together with young neighbor trees can incorporate released nutrients to their own biomass.

Wood quality can be characterized by different variables such as wood density, diameter, length, C:N ratio, lignin concentration, moisture, distance to soil etc. (Rajala et al., 2011). All of them can potentially influence rate of decomposition as

well as microbial community composition. Particularly, amount of remaining mass, relative wood moisture, pH and C:N ratio together with strong impact of tree species were identified as important factors for shaping bacterial community composition in decaying deadwood (Hoppe et al., 2015). During decomposition, the C:N ratio and wood density decrease, while moisture and lignin content increase (Rajala et al., 2011). This can have important consequences considering that the change in relative proportion of nitrogen in substrate plays decisive role in shaping microbial community (Hoppe et al., 2015). Microorganisms are partly responsible for this increasing proportion (Valášková et al., 2007) as fungi and bacteria accumulate and immobilize nitrogen through the decomposition while carbon is depleted from the biomass due to respiration. Moreover, there is the suspicion that deadwood can be enriched in nitrogen by its translocation from soil by cord-forming fungi (Laiho & Prescott, 2004).

Considering the shape of a decomposing trunk it seems that patterns of log collapse are common and repetitive among independent trunks. During the disintegration, cross-sectional width of a log remains stable and unchanged even in later stages. On the other hand, cross-sectional height of a log decreases throughout the disintegration as this process advances (Fraver et al., 2013). Resulting elliptical shape is consequence of log's vertical collapse upon itself. Activities of saprotrophic microorganisms and also macroorganisms are responsible for disruption of internal core of a log and therefore enable collapse. However, disintegration can be slowered by cover of bryophytes (Hagemann et al., 2010). Ratio of current height:current width of a log can be used for estimating the amount of remaining volume as was confirmed by Fraver et al., (2013). This estimate can serve for the prediction of amount of biomass or carbon in forest ecosystems.

Deadwood can be distinguished according to its size (i.e. diameter or volume) which shows significant influence on the course of decomposition. Deadwood volume was important driver of bacterial community composition in beech deadwood (Hoppe et al., 2015). Bässler et al., (2010) studied decomposition in the Bavarian Forest National Park and they used discrimination into two groups of deadwood similar to that used in the deadwood experiment in this thesis: coarse woody debris (CWD) and fine woody debris (FWD). Influnece of diameter together with micro- and macroclimate and resource availability to the fungal community (represented in their

study by fruiting bodies). Results suggested that abundances of fungal species on both CWD and FWD were driven by characteristics of the resource (surface area of deadwood, decomposition stage, proportion of deadwood with ground contact, etc.). Conversely, variables influencing community composition differed among CWD and FWD: CWD community composition was influenced by resource characteristics while FWD fungal community was influenced mainly by microclimate variables (exposition to sunlight, soil moisture, herb and shrub cover, etc.). Macroclimate variables (temperature, precipitation, global radiation, humidity, etc.) seemed to be important but exceeded by local variables such as microclimate and resource. As predicted, there was positive correlation between richness of species and diameter of wood due to the fact that CWD represents simply more space and can provide more niches for potential colonization. Lying logs of larger surface also offers larger area for stochastic interception of spores – phenomenon known from island biology (Lomolino, 1990). Described processes can be valid not only for fungi but also for bacteria, however, more studies are necessary for unveiling similar patterns.

3.5 Species richness interlinked with ecosystem functioning

Following short section is devoted to species richness although experiments in this thesis did not deal with diversity manipulation. Richness of the community in discussed habitats was changing over time and this shift likely had impact on functioning of community. Generally, diversity during any succession increases. This increase may has interesting consequences. Namely more diverse community probably exploits broader spectrum of resources instead of competing and enables cross-feeding. Due to this “complementarity effect” (Wohl et al., 2004) community can be more productive in total. Another hypothesis counts with partial redundancy of substrate preferences by functionally similar taxa resulting in facilitation of degradation via diffusible enzymes (Hättenschwiler et al., 2011). This redundancy also provides stability of particular habitat ensuring maintenance of its functioning after disturbances. Interestingly, functional redundancy (approached via enzymatic activities) was observed during decomposition of litter in structurally different microbial communities in forests with various management practices (Purahong et al.,

2014). Higher richness can further enable degradation of inhibitors as products of one group and substrates of another group of microorganisms. “Selection effect” (Bell et al., 2005) as another pattern describes higher probability of presence of groups with large effect on ecosystem in a diverse community. However, higher rate of antagonism in rich ecosystems can arise as well. Therefore, species richness is not universal predictor of rate of substrate utilization.

3.6 Microbial relationships in the substrate

Leaf litter and deadwood are habitats containing sources of nutrients and substrate for colonization. Also can represent the platform for microbial interactions and for build-up of complex relationships through either physical contact or enzyme production (Velicer, 2003). In these environments, where microorganisms have to strive to survive strong competition, relationships are established even between phylogenetically distant organisms. Inevitably these are also bacteria and fungi. They have to face with rapid labile nutrients depletion as well as limited numbers of free niches.

Relationship between fungi and bacteria as dominant saprotrophic organisms can take various forms (Boer et al., 2005). It seems that development of competition (Møller et al., 1999), antagonism (Mille-Lindblom & Tranvik, 2003; Schneider et al., 2010) or cooperation depends on conditions (such as pH, moisture, substrate structure) and type of resources (plant biopolymers, oligosaccharides, microbial biomass, metabolites, aromatic compounds). Fungal influence modulating abundances and community composition of bacteria colonizing deadwood in soil microcosm is described as well (Folman et al., 2008). Moreover, predominance of fungal enzymes in leaf litter was detected (Schneider et al., 2012). Under such a evolutionary pressure, bacteria in some cases have developed strategies how to surpass fungal neighbors and how to benefit from their presence.

These situations when bacteria use nutrients released by fungal action can be considered as “cheating behaviour” (Velicer, 2003). Due to the diffusible character of fungal enzymes secreted into substrate, products of enzymatic cleavage are accessible to all consumers in the vicinity including producer fungi and cheating bacteria. This

phenomenon was probably responsible for better growth results of *Pectobacterium carotovorum* (plant-pathogenic *Gammaproteobacterium*) in co-culture with *Aspergillus nidulans* (model ascomycete) where bacterium used products of fungal metabolism (Schneider et al., 2010).

Mentioned experiment of Schneider et al., (2010) with co-culture of *P. carotovorum* and *A. nidulans* continued and surprisingly turned out into form of antagonism when the bacterium negatively influenced the fungus by faster growth. Antagonistic influence may not be only one-directional. Bilateral negative influence after coinoculation of *Phragmites* biomass in aquatic environment by fungi and bacteria has been described (Mille-Lindblom & Tranvik, 2003). In this study, fungal strains and bacteria displayed inhibition of growth caused by the presence of each other. Notably, sensibility of microorganisms against competitor's inhibition does not seem to be constant during the competion. Sensibility can be modulated by energy investment into development of mechanisms for tolerance (Mille-Lindblom et al., 2006). Therefore, this modulation has form of trade-off between capability for tolerance and sustainable growth. Obviously another example of negative influence of bacteria is production of antibiotics.

Coexistence of fungi and bacteria may not necessarily take the form of competition. One could imagine situation when presence of fungi can create niches or provides access to nutrients for bacteria (Valášková et al., 2009). Metabolism of fungi is responsible for acidification of wood by secretion of metabolic byproducts such as oxalic acid (Clausen, 1996; Boer et al., 2005). Although some bacteria can protect themselves as they are capable of degrading acidic fungal exudates (Schoonbeek et al., 2007), low-pH conditions may not be favourable for all bacteria. Therefore, it can provide competitive advantage for acidotolerant taxa as those of the phylum *Acidobacteria* (Valášková et al., 2009) whose abundance increases with decreasing pH as was described in soil (Jones et al., 2009).

Another form of interaction occurring at microbial level of coexistence is potential ability to attack fungal hyphae by mycophagous bacteria. Saprotrophic life on dead fungal bodies which represent an important portion of total biomass in particular microenvironments is next possible strategy (Fig. 4). While in the case of direct attack on live fungi it is still unclear how widespread this phenomenon is (De Boer et al.,

2005), the dead fungal biomass is significant source of nutrients (mainly of valuable nitrogen) and its exploitation by mycophagous bacteria is probably frequent. Interestingly, the reverse situation is also possible – bacterial colonies may represent source of nitrogen for certain fungi (Tsuneda & Thorn, 1995). Decomposition of fungal body is possible due to enzymatic apparatus consisting of chitinases, glucanases or proteases. To be concrete, novel genus *Collimonas* was described for its ability to grow on living fungal biomass in soil (De Boer et al., 2004). Further mycolytic activity was reported for the myxobacteria – group known for its social behaviour (Homma, 1984), *Paenibacillus* (Dijksterhuis et al., 1999) and also for *Pseudomonas* connected with brown blotch disease of *Agaricus bisporius* (Jolivet et al., 1999).

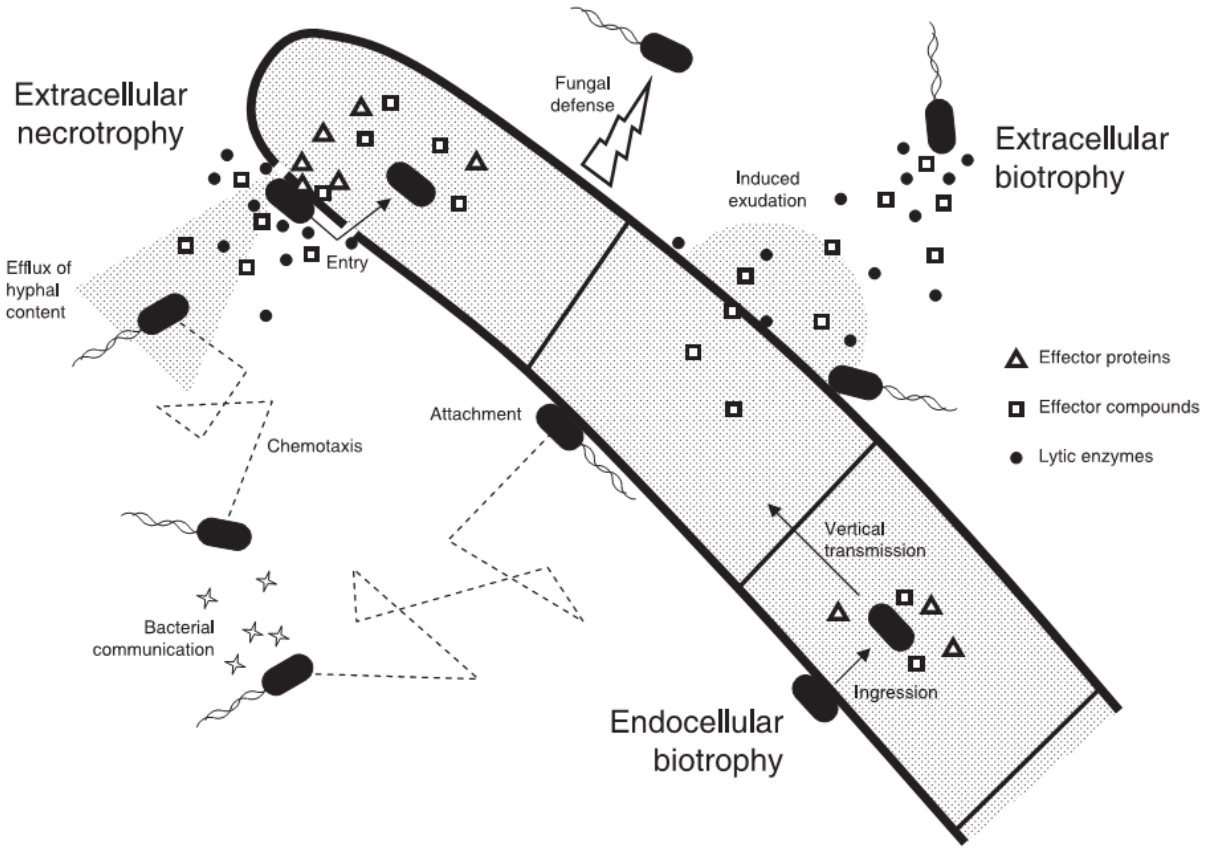


Fig. 4: Scheme of possible bacterial interactions with fungal hyphae encompassing extra- and endocellular interactions. (adapted from Leveau & Preston, 2008)

Even though not all bacteria are mycophagous, they still can physically interact with fungi and stick to their mycelium (Folman et al., 2008). Fungal hyphal cords are

ideal for penetrating of substrate while bacterial movement in a compact substrate or substrate with air-containing pores such as wood, litter or soil may be limited. Hence, hyphae can serve as a path for transport allowing overcoming of air-filled cavities or other barriers in substrate. Similar migration along mycelium revealed relations between bacterial groups resembling hitchhikers on fungal mycelium (Warmink et al., 2011).

Annual periodicity of litter and, to some degree, periodicity of death trees allow to develop specific colonization strategies for successful substrate occupation and for acqusition of advantage in competition. So called priority effect (Hiscox et al., 2015; Mille-Lindblom et al., 2006) means that microorganism or group of microorganisms colonize habitat and its niches before others. It can provide better starting position for already established taxa allowing them to outcompete others and succeed during initial phases of decay. Priority effect plays important role in microbial succession after senescence of plant biomass by establishment dominant community members yet before leaf abscission (accounting for phyllosphere microorganisms) or before death of a tree. The predominance of endophytic fungi over bacteria was observed on oak litter (Šnajdr et al., 2011) and these fungi persisted after leaf abscission and could have played important role during the decomposition (Voříšková & Baldrian, 2013).

Microbial interactions in decomposing plant biomass have already revealed some of their principles although their systhematic exploration was not yet undertaken. Other gaps of the present knowledge, already beyond the scope of this thesis, are for example the uncertainity whether carbon transfer between microorganism is possible (Paterson et al., 2008) or whether there are mutualistic interactions between wood-decomposing fungi and methylotrophic N-fixing bacteria (Hoppe et al., 2014).

4 Materials and methods

4.1 Succession of bacterial community on leaf litter

Section 4.1 is devoted to the experiment that focused the bacterial community associated with *Quercus petraea* leaves and litter in the conditions of the forest in Central Europe. The aim was to describe the successional changes in the composition of the community using sequencing of DNA and to explain the occurence and dynamics in the abundances of some bacterial taxa as a result of changes in the substrate composition and nutrition availability.

The same samples of decomposing leaf litter were used also in the study of Šnajdr et al., (2011) where the enzyme activities, quantification of fungal/bacterial biomass and chemical characterization of leaf litter was published. Isolated environmental DNA was used for description of fungal community development in the study of Voříšková & Baldrian, (2013).

4.1.1 Study site

The study site was a sessile oak (*Quercus petraea*) forest in the Xaverovský háj Natural Reserve (established in 1982), near Prague, Czech Republic (50° 5' 38" N, 14° 36' 48" E). This site has been targeted in many previous studies focused on activity of decomposition-related enzymes in the forest topsoil (Baldrian et al., 2013; Šnajdr et al., 2008), activity of these enzymes during the successive decomposition of *Q. petraea* litter (Šnajdr et al., 2011), saprothrophic fungi were characterised in this site (Šnajdr et al., 2011; Valášková et al., 2007). The composition of fungal community associated with leaf litter decomposition was explored (Voříšková & Baldrian, 2013) and seasonality changes in fungal and bacterial community composition was studied (López-Mondéjar et al., 2015; Voříšková et al., 2014).

The soil was an acidic cambisol with litter (L), organic horizon (O), and the mineral horizons Ah and A. Litter thickness was 0.5-1.5 cm, with average pH 4.3, 46.2% of C content, 1.76% of N content. The mean winter temperature at the soil

surface was 1.3°C, mean summer temperature was 16.6°C and mean annual temperature was 9.3°C. Weekly minimal and maximal temperature for L horizon was 0.2°C and 15.7°C (Baldrian et al., 2013).

4.1.2 Sampling of litter

The experiment was performed by incubating litterbags *in situ* as described previously (Šnajdr et al., 2011; Voříšková & Baldrian, 2013). Leave litter of *Q. petraea* (tree age 100-120 years) for litterbag construction was collected immediately after abscission and allowed to air dry at 20°C. Litterbags containing 5 g of air-dried leaves (10 × 20 cm, 1 mm nylon mesh size) were placed on the top of the litter horizon at the study site at the end of the litterfall season (November). To prevent extensive desiccation, litterbags were overlaid with freshly fallen oak leaves. Four replicate litterbags were removed for DNA extraction after 2, 4, 6, 8, 10, 12, 18 and 24 months of incubation.

For the analysis of the phyllosphere bacterial community composition, live *Q. petraea* leaves were collected two months before abscission by hand-picking (month -2) and senescent leaves were collected during the litterfall period by gently shaking oak twigs and collecting falling leaves before their contact with the soil (month 0).

Collected material was transferred to the laboratory and processed immediately. Litterbags were opened and material was cut into 0.25 cm^2 pieces and used immediately for DNA extraction. The same material was also used for chemical analyses, measurement of enzyme activities and quantification of microbial biomass as described in Šnajdr et al., (2011).

4.1.3 DNA extraction and PCR

The total genomic DNA was extracted from 300 mg of material using the Powersoil® Kit (MoBio, Carlsbad, USA). This extracted DNA was also used for determining composition of fungal community in litter (Voříšková & Baldrian, 2013).

The primers 515F and 806R (Caporaso et al., 2011) were used to amplify hypervariable region V4 of the 16S rRNA gene (Fig. 2).

Primer 515F: 5'- GTGCCAGCMGCCGCGGTAA -3'

Primer 806R: 5'- GGACTACHVGGGTWTCTAAT -3'

These primers are degenerated to ensure better coverage of uncultivated microorganisms. The sequence of the primers is listed according to IUPAC nucleotide code. Primers were developed for sequencing of bacterial communities from different habitats. Although amplification with these primers yields relatively short amplicons (250 bp) they proved to be sufficient for optimal clustering and identificaton of obtained sequences during following analysis (Liu et al., 2007).

Each forward primer was barcoded by a sequence of 4-6 nucleotides designed to facilitate multiplexing of different samples and for their simple filtering during data analysis.

PCR amplification was performed in the GenePro Thermal Cycler (Bioer, Hangzhou, China) using modified protocol described by (Caporaso et al., 2011). PCR was performed in triplicates and every reaction contained following components:

2.5 μl 10x buffer for DyNAzyme DNA Polymerase (Thermo Fischer Scientific)
0.75 μl DyNAzyme II DNA polymerase (2 u μl^{-1}, Thermo Fischer Scientific)
1.5 μl BSA (10 mg ml^{-1}, GeneON)
0.5 μl PCR Nucleotide Mix (10 mM, Bioline)
0.5 μl primer 515F (10 μM, Sigma Aldrich)
0.5 μl primer 806R (10 μM, Sigma Aldrich)
1.0 μl template DNA, concentration approx. 5-50 ng μl^{-1}
sterile ddH_2O added up to 25 μl

Conditions for amplification were:

1. initial melting: 94°C - 4 minutes
2. melting: 94°C - 45 seconds
3. annealing: 50°C - 60 seconds
4. extending: 72°C - 75 seconds

5. final extending: 72°C - 10 minutes
6. holding: 4°C

Step 2-3 was repeated 35 times.

Successful PCR amplification was confirmed by gel electrophoresis with 1% agarose gel. Triplicate PCR reaction products of each sample were than joint together and cleaned and concentrated using MinElute PCR Purification Kit (Qiagen, Hilden, Germany). DNA was eluted from the column by 20 μl of EB buffer (10 mM Tris/HCl, pH 8.5).

Concentration of DNA in each sample was measured using the Qubit® 2.0 Fluorometer (Life Technologies, Waltham, USA) using dsDNA High Sensitivity Assay Kit. Different PCR products were then mixed in an equimolar ratio to obtain the same amount of DNA from each sample.

4.1.4 Ligation of adapters

DNA concentration in the composite sample which contained different amplicons with diverse barcodes was further meausured on Qubit® 2.0 and an aliquot containing 1 μg of DNA was taken and its volume was brought to 50 μl by adding EB buffer. This volume is suitable for ligation of sequencing adaptors.

Sample was processed according to the modified Illumina Paired-End Sample Preparation Protocol using the TruSeq DNA PCR-Free LT Kit (Illumina, San Diego, USA). After getting through the steps of the workflow two adaptors are ligated to the ends of amplicons (Fig. 5). The first step presents treating by mixture of polymerases with 3'→5' exonuclease activity and 5'→3' polymerase activity. It provided blunt DNA ends. Amplicons with insufficient length were removed using Agencourt AMPure XP (Beckman Coulter, Miami, USA). After purification, DNA was eluted by 20 μl of EB buffer. 5' ends were than phosphorylated and 3' ends were adenylated. Finally, paired-end adaptors were ligated using the 3'- dA overhang.

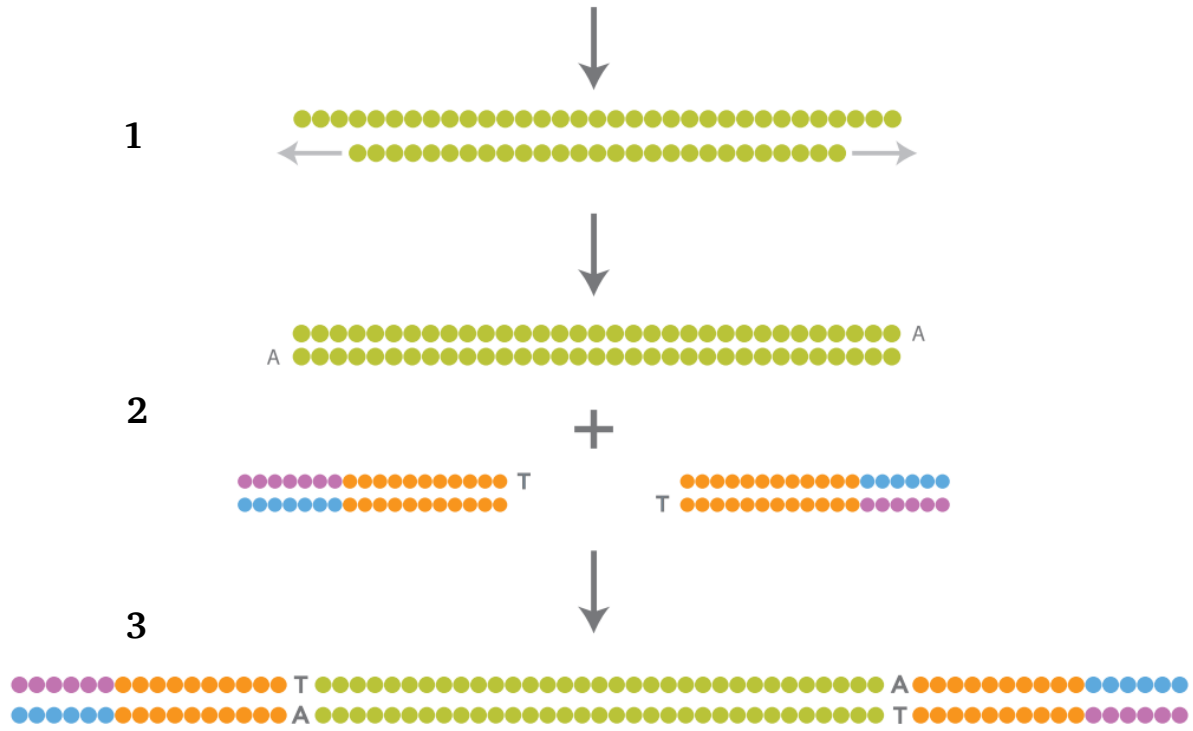


Fig. 5: Ligation of adaptors to amplicons prior to Illumina sequencing. 1- Creating of blunt ends. 2- Adding of adenin to 3' ends and ligation of adapters. 3- Final product. (Genome Analyzer Brochure, 2010)

4.1.5 Illumina MiSeq sequencing

Quantification of DNA was done using the KAPA Library Quantification Kit Illumina (Kapa Biosystems, Wilmington, USA) on the Step One Plus™ Real-Time PCR System (Applied Biosystems, Waltham, USA).

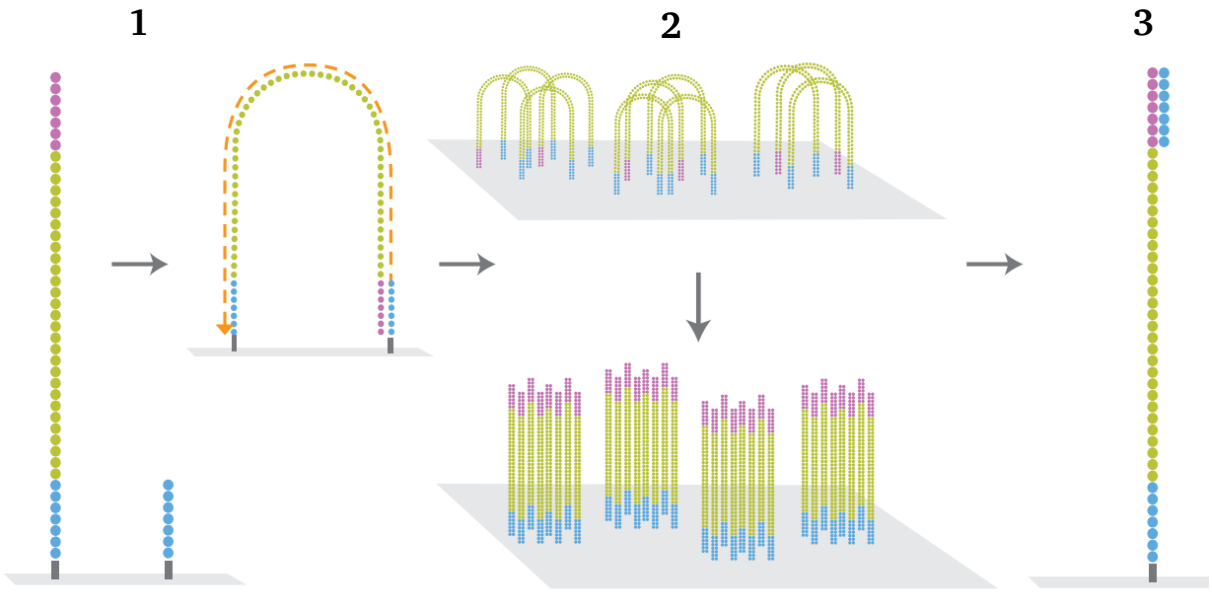
The amplicons were sequenced on the Illumina MiSeq platform (Argonne National Laboratory, Lemont, USA). This platform was introduced in 2006 and allows to sequence metagenomic samples with low error rate at sufficient sequencing depth and good cost/Mb ratio (Glenn, 2011). This method is based on sequencing by synthesis and it uses reversible termination by nucleotides labelled with four different fluorescent dyes (Fig. 6).

First, DNA sample is denatured and it is aplicated to a surface ("flow cell") which is covered by oligonucleotides complementary to the adapters at the ends of amplicons of our interest. ssDNA is immobilised this way. In the conditions that favour PCR cycling and with PCR reagents, each amplicon creates "bridge" by hybridization

between its free end and complementary sequence on the surface. Complementary adapters on the surface act as primers for PCR. After that one strand of DNA is removed. As a result clusters of roughly 1 000 ssDNA copies (so called “polonies”) are created in a flow cell. This step is essential for sufficient light signal intensity during later addition of bases.

After creation of clusters, reagents for DNA synthesis are added in a flow cell. These are DNA polymerase, primers and four bases labelled by diferent fluorescent dyes which allow reversible termination. After incorporation of base into the DNA strand fluorescent signal and its position is detected by the sensitive CCD chip. Reversible terminator at the 3'-end as well as fluorescent dye is than removed and incorporation of the next base can be repeated. Aforementioned method has sufficient sequencing depth for metagenomic studies. Tens of millions of clusters can be sequenced in parallel.

This method allowed acquirement of sequences of 16S rRNA gene from bacterial community associated with leaf litter.



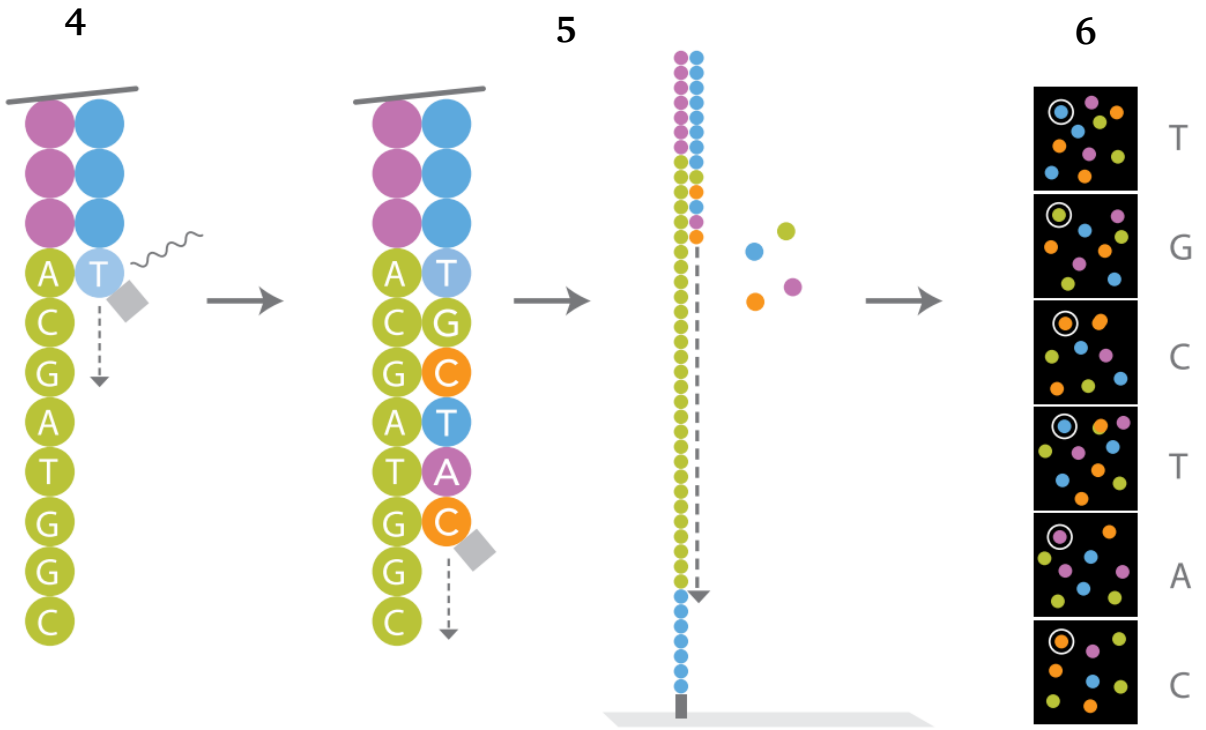


Fig. 6: Preparation of immobilized DNA of our interest and principle of Illumina sequencing. 1- Immobilization of amplicon on the surface and its bending which creates a bridge. 2- PCR creates "polonies" (clusters of identical DNA copies). 3- One DNA strand is removed resulting in ssDNA clusters. 4- Complementary nucleotide with reversible terminator begin polymerization of the second DNA strand. After incorporation of a nucleotide fluorescent signal specific for each base is emitted. 5- Cycling of incorporation and terminantor removal allows strand extension. 6- Fluorescent signals are recorded together with their position in a flow cell resulting in nucleotide sequence. (adapted from Genome Analyzer Brochure, 2010)

4.1.6 Bioinformatic analysis

The sequencing data were processed using SEED (Větrovský & Baldrian, 2013). Paired-ends of raw fastq files were joint together by Fastq-Join tool (Aronesty, 2013) with minimum overlap of 40 bp and 15% of maximum difference. Sequences containing unspecified bases ("N" in the nucleotide sequence) were omitted as well as sequences with quality mean below 30. Chimeric sequences in the dataset were detected and removed by UCHIME algorithm (Edgar et al., 2011) which is part of the USEARCH package. After chimera removal sequences shorter than 200 bp were omitted.

Sequences in the dataset were clustered by UPARSE clustering algorithm (Edgar, 2013, also part of the USEARCH package) at 97% similarity level to receive Operational Taxonomic Units (OTUs) that correspond to bacterial taxa approximately at the level of species (Konstantinidis et al., 2006). Consensus sequences were constructed for all OTUs using MAFFT algorithm (Katoh et al., 2005). All sequences in each OTU or 250 randomly chosen sequences for OTUs with >250 sequences were used for creating consensus sequences. Global alignment G-INS-i was used for obtaining pairwise alignments and gaps were cleaned from consensus.

Resulted consensus sequences were searched against the downloaded NCBI nucleotide database for 16S rRNA gene (database version from September 2014) using BLASTn algorithm specifically MegaBLAST (Morgulis et al., 2008) with a similarity threshold (E-value) of 0.00001. For best database hits, phylogeny was retrieved from GenBank based on their accession numbers.

4.1.7 Measuring of enzyme activities and microbial biomass

Description of measurement of enzyme activities and microbial biomass was performed in the frames of the previous experiment using the same samples as described by (Šnajdr et al., 2011). To briefly summarize the used techniques, endo-1,4-β-glucanase and endo-1,4-β-xylanase were measured with azo-dyed substrates carboxymethyl cellulose and birchwood xylan, respectively. Protocol of the supplier was used (Megazyme, Ireland). One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol reducing sugars per minute. Exocellulase activity was measured in microplates using *p*-nitrophenyl-β-D-cellobioside. Laccase activity was measured as oxidation of 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid in citrate-phosphate buffer. Manganese peroxidase activity was measured in succinate-lactate buffer in which product of oxidative coupling of methyl-2-benzothiazolinone hydrazone and 3,3-dimethylaminobenzoic acid was detected spectrophotometrically. Activities of 1,4-β-glucosidase (EC 3.2.1.21), 1,4-β-xylosidase (EC 3.2.1.37) and 1,4-β-*N*-acetylglucosaminidase (EC 3.2.1.52) were measured using *p*-nitrophenyl-β-D-glucoside, *p*-nitrophenyl-β-D-xyloside and *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, respectively. All spectrophotometric

measurements were carried out in a microplate reader (Infinite, Tecan) or a UV-VIS spectrophotometer (Lambda 11, Perkin-Elmer).

Bacterial biomass was quantified as the sum of the following phospholipid fatty acids: i14:0, i15:0, a15:0, 16:1ω7t, 16:1ω9, 16:1ω7, 10Me-16:0, i17:0, a17:0, cy17:0, 17:0, 10Me-17:0, 10Me-18:0 and cy19:0. Fungal biomass was quantified based on 18:2ω6,9 content. The free methyl esters of PLFAs were analysed by GC/MS (Varian 3400; ITS-40, Finnigan).

4.1.8 Diversity indices

Diversity indices were calculated in SEED (Větrovský & Baldrian, 2013). There are more ways to assess diversity in a community. Most of them have been developed for the macroecology but are applicable in the microbial ecology as well. For assessment of diversity, each sample was randomly resampled to 1 500 sequences to eliminate the effects of dissimilar sampling depth.

Rarefaction curves (Hughes et al., 2001) were obtained by averaging randomizations of the observed accumulation curves.

The formula for rarefaction is:

$$S_n = S_t - \left(\frac{\sum_{i=1}^{S_t} \binom{N - N_i}{n}}{\binom{N}{n}} \right)$$

S_n = average number of OTUs observed after drawing n sequences

S_t = total number of OTUs in samples of N total sequences

Another approach is to estimate total richness of a community from a sample. Several estimates of community richness were used and richness can be compared across samples. Microbial ecology can adapt various richness estimators but nonparametric estimators seem to be most promising (Hughes et al., 2001). These are based on mark-release-recapture strategy widely used in ecology. They consider ratio between OTUs observed only once to OTUs that have been observed before (i.e. “recaptured” OTUs). The idea behind this approach is that in a very diverse

dataset the probability of observing particular OTU more than once is lower than in a uniform dataset.

Chao1 (Chao, 1987) is one of the nonparametric estimators which is suitable for microbial datasets because they often contain a lot of low-abundance OTUs (Chao, 1984). On the other hand, Chao1 yields biased true richness estimates in undersampled datasets. This estimator calculates with number of OTUs consisting of only one sequence (singletons) and of only two sequences (doubletons). In accordance with principles of nonparametric estimators described above, Chao1 calculation considers the hypothethical situation when there are all OTUs in a dataset observed at least twice. It means that there are no other undiscovered OTUs and total richness estimate can be calculated.

Formula for Chao1 richness estimate is:

$$S_{Chao1} = S_{obs} + \frac{n_1^2}{2n_2}$$

S_{obs} = the number of observed species

n_1 = the number of OTUs with only one sequence (i.e. singletons)

n_2 = the number of OTUs with only two sequences (i.e. doubletons)

Diversity was also expressed by the Shannon-Wiener Diversity Index (H') (Shannon, 1948). This index includes both species richness and evenness of species abundance (Colwell, 2009). It was originally developed for describing entropy in strings of text. Adapted by ecology, it quantifies the uncertainty in predicting the species identity of an individual that is taken at random from the dataset. Its formula is:

$$H' = -\sum_{i=1}^{R} p_i \, ln \; p_i$$

p_i = number of individuals of species *i* divided by total number of samples

Formula for Evenness (E) which is related to the Shannon-Wiener Index is:

$$E = \frac{\mathrm{H}'}{\mathrm{H}'_{max}}$$

H'_{max} = ln of total number of species

4.1.9 Statistical analysis

One-way ANOVA followed by the Fischer's LSD post-hoc test was used to test for statistical differences among groups in Statistica 7.0 (StatSoft, Tulsa, USA). Differences at $p < 0.05$ were regarded as statistically significant. The influence of factors on community composition was analysed by Principal Component Analysis (PCA) in Statistica 7.0. PCA analysis was based on square root (normalization of data) of relative abundances of the 54 most abundant genera which showed abundances $\geq 0.5\%$ in at leat 5 samples. Months, wood properties and enzyme activities were used as environmental variables and the relative abundances of genera were used as species variables. Similarities between communities were analysed by ANOSIM (Clarke, 1993) using Bray-Curtis similarity matrix (9 999 permutations). Correlation between community composition and enzyme activities were analysed by Mantel test (Mantel & Valand, 1970) using Bray-Curtis and Euclidean matrices for abundances of OTUs and values of enzyme activities, respectively. ANOSIM and Mantel test were performed using PAST 3.05 (Hammer et al., 2001). Differences at $p < 0.05$ were regarded as statistically significant.

4.2 Bacterial community in deadwood

The second experiment that was established in 2012 in the Bavarian Forest National Park, Germany was aimed to describe the successive colonization of deadwood by bacteria considering tree species (fir – *Abies alba*, beech – *Fagus sylvatica*) and deadwood size (diameter) as the most important variables.

Isolated environmental DNA from the same samples of deadwood from the year 2012 was used for description of fungal community and its composition by Zrůstová, (2014).

4.2.1 Study site

Several transects in Bavarian Forest National Park were chosen as the study site for this experiment which was performed in cooperation with the group of Claus Bässler,

Bavarian Forest National Park. The area where deadwood was located was approximately 25 000 ha large and lied in south-eastern Germany with altitude ranging from 650 to 1 450 m a. s. l. Mean annual temperature varied from 3.5 to 7.0°C and it was dependent on elevation. Total annual precipitation ranged from 1 300 to 1 900 mm. The amount of deadwood in the national park has increased in the last 25 years significantly due to bark beetle, windthrows and banned logging to densities exceeding 700 m^3 ha^{-1} (Bässler et al., 2010) and deadwood thus represents an important niche in the ecosystem.

4.2.2 Sampling of decomposing wood

Newly cut logs or branches of fir and beech were laid at >120 sites across the study area in 2011, each site containing either 10 logs or 20 branches. Collection of samples was performed after one year and two years of decomposition (2012 and 2013). Each year, 62 branches of diameter up to 5 cm (fine woody debris, FWD) and 62 logs of approximately 30 cm diameter (coarse woody debris, CWD) were sampled. Half of logs and branches was from beech and half was from fir.

Samples of FWD at each site were obtained from four branches using electric drill. Two drillings were performed on each branch and these drilling spots were 40 cm from each other. Four samples of CWD were obtained from each log. Drilling spots were at 1/8, 3/8, 5/8 and 7/8 of the log length in 2012 and at a 20-cm distance from these drilling holes in 2013. Two and two drills within a log were merged for DNA extraction. Deadwood was drilled without surface bark, drill was sterilized by washing in ethanol between drilling and woody material was collected to plastic bags. Samples were frozen and transported to the laboratory in Prague on ice. In the laboratory, material was weighted, freeze-dried and than weighted again to estimate the dry mass content of wood. Material was than milled using Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany) and the resulting fine sawdust was used for analyses.

4.2.3 DNA extraction and PCR

Total genomic DNA was extracted from 150-200 mg of material using NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany) according to the manufacturers instructions: cells were lysed by SL1 lysis buffer. Enhancer SX was added before lysis. Samples were homogenized using FastPrep®-24 (MP Biomedicals, Santa Ana, USA) at 5 m s^{-1} for 2× 30 sec. In the last step, DNA was eluted from columns using 50 μl of elution buffer SE (5 mM Tris/HCl, pH 8.5). Two replicate extractions per sample were performed. The same DNA samples were also used for the analysis of fungal community composition. Data from year 2012 were published by Zrůstová, (2014).

For PCR amplification, similar setups of barcoding, composition of reaction mix and cycling conditions were used as in Experiment 1 (section 4.1.3) together with similar DNA purification.

4.2.4 Ligation of adapters and Illumina MiSeq sequencing

Ligation of adapters followed the same procedure which is described in the section 4.1.4.

Sequencing was done in external laboratories using Illumina MiSeq sequencing platform. Samples from year 1 were sequenced in the GeneTiCA, Brno, Czech Republic. Samples from year 2 were sequenced in the Argonne National Laboratory, Lemont, USA.

4.2.5 Chemical properties and enzyme activities

pH was measured after mixing with distilled water (1:10 w/vol). Content of carbon and nitrogen in samples was measured in the Research Institute for Soil and Water Conservation (Prague, Czech Republic).

For enzyme activity analysis, freeze-dried samples were extracted at 4°C for 2 hours on an orbital shaker (100 rpm). 12 ml of acetate buffer were used for extraction from 250 mg of each sample. Substrates with bound fluorescent dyes

(4-methylumbellyferol – MUF and amidomethylcoumarin – AMC) were incubated with sample extracts and fluorescence was measured on a plate reader Infinite (TECAN, Austria) to infer activity of β-glucosidase, *N*-acetylglucosaminidase and exocellulase. 40 μl of each substrate was added to microtitter plate together with calibration dilution. 200 μl of extract was added to substrates. Plate was than incubated at 40°C. Spectrophotometrical measurement was done after 5 and 125 minutes (excitation wavelength – 355 nm, emission wavelength – 460 nm).

Activity of endocellulase and endoxylanase was measured using azo-dyed carboxymethylcellulose and xylan (Megazyme, Ireland). 150 μl of sample and 150 μl of substrate was mixed and than incubated in 40°C for 2 hours. Incubation was stopped by adding 750 μl of ethanol, vortexing (10 s) and centrifugation (10 000 g, 10 min). Measuring was performed at wavelength 595 nm (Baldrian, 2009).

Activity of laccase and manganese peroxidase was assayed using the same principles as in the paper (Šnajdr et al., 2011). In the case of laccase measurement, 150 μl of citrate-phosphate buffer, 50 μl of extract and 50 μl of 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid was mixed and measured at wavelength 420 nm.

4.2.6 Bioinformatic analysis

Bioinformatic analysis followed the same pipeline as described in the section 4.1.6. However after taxonomy assignment to the OTUs, nonbacterial sequences were omitted. Further, both datasets (year 1 and 2) containing only bacterial sequences were merged and clustered into OTUs at the 97% threshold. Samples with sequence counts higher than 10 000 were randomly resampled and reduced to 10 000 sequences per sample because of better processing of the algorithm for creating consensus sequences. Taxonomy assignment was than performed with the same settings as in the first round before omitting non-bacterial sequences.

4.2.7 Diversity indices and statistical analysis

Values of diversity were counted similarly as decribed in the section 4.1.8 using SEED pipeline.

Diversity indices were counted from randomly resampled data to the 1 000 sequences per sample. Estimates were than averaged within deadwood type. Statistical analysis followed esentially the same steps as described in the section 4.1.9. Indicator species analysis was performed using indicspecies package (De Caceres & Legendre, 2009) in R (R Core Team, 2015). In this analysis, *specificity* stands for predictive value of the OTU as indicator for the site group, *fidelity* is probability of finding the OTU in particular site group. Hence, OTU with *specificity*=1 in FWD/T was found only in this site group, while *fidelity*=0.5 means that was present in one half of samples within this site group.

5 Results

5.1 Bacterial community on *Quercus petraea* leaves and litter

After MiSeq sequencing and quality filtering 12 875 OTUs comprising 388 267 sequences (>200 bp) were available for analysis. BLASTn against local 16S rRNA database (version from 15. 9. 2014) found hits for 12 030 OTUs (93.4%). From these clusters 11 491 OTUs (298 175 sequences, 89.3% from total OTUs) were assigned as bacterial and were used for further analysis (Table 1, Fig. 7). Other sequences were mostly assigned to the kingdom *Viridiplantae* (derived from *Q. petraea* chloroplasts).

		Months									
	total	-2	0	2	4	6	8	10	12	18	24
number of samples	37	3	3	3	4	4	4	4	4	4	4
number of sequences	298 175	1 930 (0.6%)	11 182 (3.8%)	51 436 (17.3%)	42 022 (14.1%)	27 699 (9.3%)	47 485 (15.9%)	15 362 (5.2%)	39 828 (13.4%)	42 896 (14.4%)	18 335 (6.1%)
mean sequences/sample	8 059	643	3 727	17 145	10 506	6 925	11 871	3 841	9 957	10 724	4 584
median sequences/sample	8 830	807	3 408	14 477	11 057	6 839	11 983	3 885	10 010	9 436	3 911
number of OTUs	11 491	206 (1.8%)	471 (4.1%)	1 262 (11.0%)	1 318 (11.5%)	1 269 (11.0%)	2 397 (20.9%)	1 971 (17.2%)	3 753 (32.7%)	2 985 (26.0%)	2 256 (19.6%)
mean OTUs/sample	718	86	196	501	444	485	885	832	1 409	1 124	875
median OTUs/sample	692	89	206	409	466	464	916	854	1 395	1 121	783
relative abundance of top* OTUs	75.9%	59.8%	71.9%	92.9%	94.3%	89.5%	80.1%	57.4%	58.3%	57.8%	58.8%

Table 1: Sequencing results from different months during two years of leaf litter decomposition. * top OTUs had relative abundances ≥ 0.5% in at least three of all samples; top OTUs are listed in the Table S1 in the Appendix.

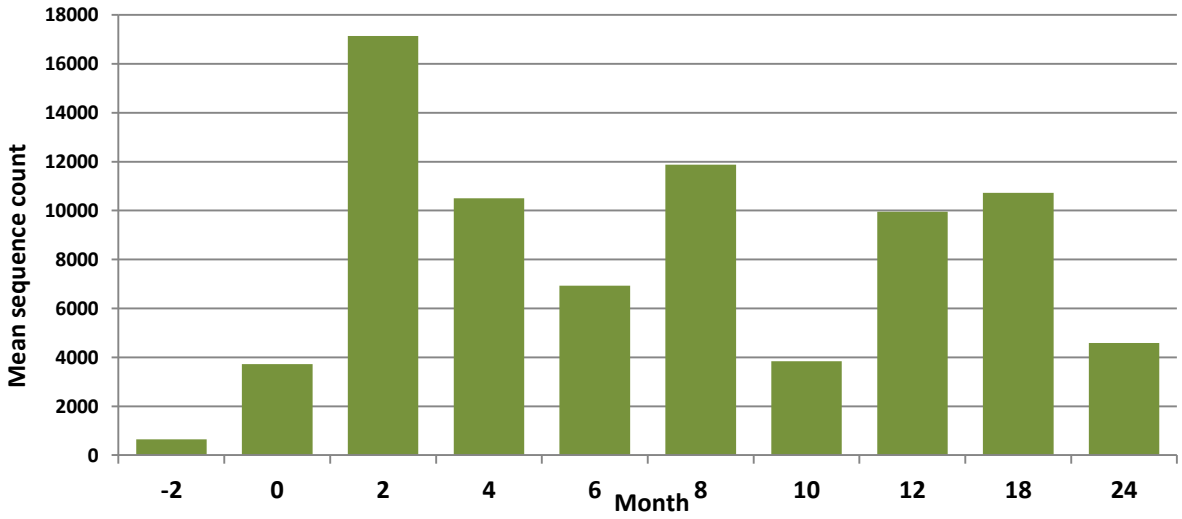


Fig. 7: Histogram of mean sequence counts after filtering obtained from 3-4 samples from each month.

5.1.1 Composition of community associated with leaves and litter

Bacteroidetes, *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* were the most abundant bacterial phyla/classes throughout the whole experiment with abundances ≥ 0.5% in all 37 samples (Fig. 8). These were followed by less abundant *Actinobacteria*, *Acidobacteria*, *Firmicutes*, *Deltaproteobacteria* and *Verrucomicrobia*. All of them represented ≥ 0.5% still in more than half of all samples.

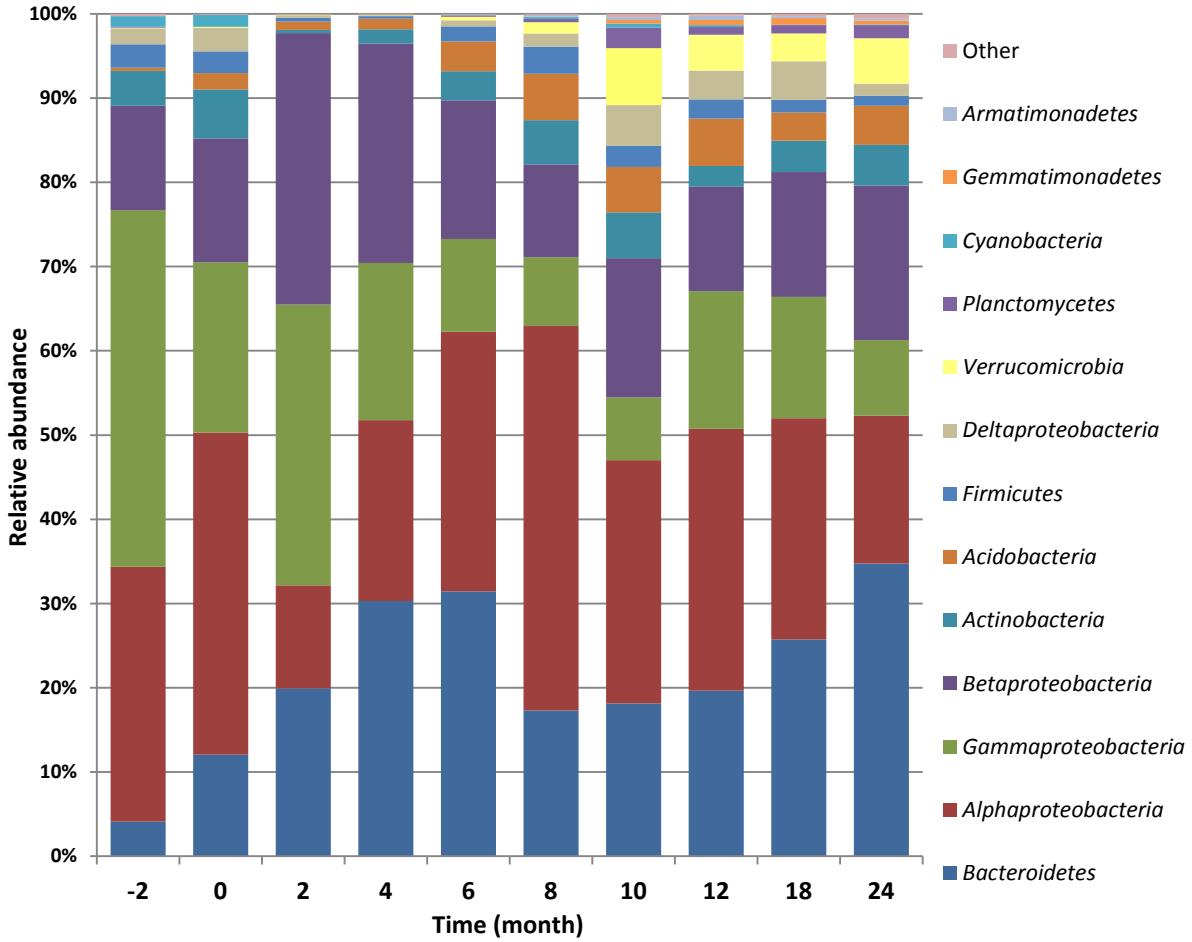


Fig. 8: Relative abundaces of bacterial phyla (or classes in *Proteobacteria*) on *Q. petraea* leaves and litter. Displayed taxa showed abundances ≥ 0.5% in at least 3 samples from the whole set of samples. Taxa with lower abundances are labeled as "Other". The data represent means of three or four replicate litterbags. Samples from Month -2 are derived from live leaves and samples from Month 0 are from senescent leaves still attached to branches.

The most abundant genera associated with leaves from month -2 were *Pseudomonas* (27.9%), *Sphingopyxis* (7.7%), *Methylosinus* (7.6%), *Acinetobacter* (6.0%) and *Duganella* (5.7%). However, only *Pseudomonas* and

Duganella showed sufficient overall abundances in following months to be displayed in Fig. 9. At the beginning of decomposition in month 2 genera *Pseudomonas*, *Sphingomonas*, *Pedobacter*, *Duganella*, *Massilia* and *Janthinobacterium* each showed abundance at least 5%. The abundances of the latter two genera peaked in this month, the other genera showed further short increase but than all of them decreased as decomposition reached its advanced phase (Fig. 10). Middle phase including months 4-8 was dominated by *Sphingomonas* and *Pedobacter*. In a phase of late decomposition from the month 10 until the end of sampling in the month 24, genera *Mucilaginibacter*, *Burkholderia*, *Paucibacter* and *Bradyrhizobium* were recorded as the most abundant. First three mentioned exceeded 5% threshold in the month 24 (Fig. 9, Table 2).

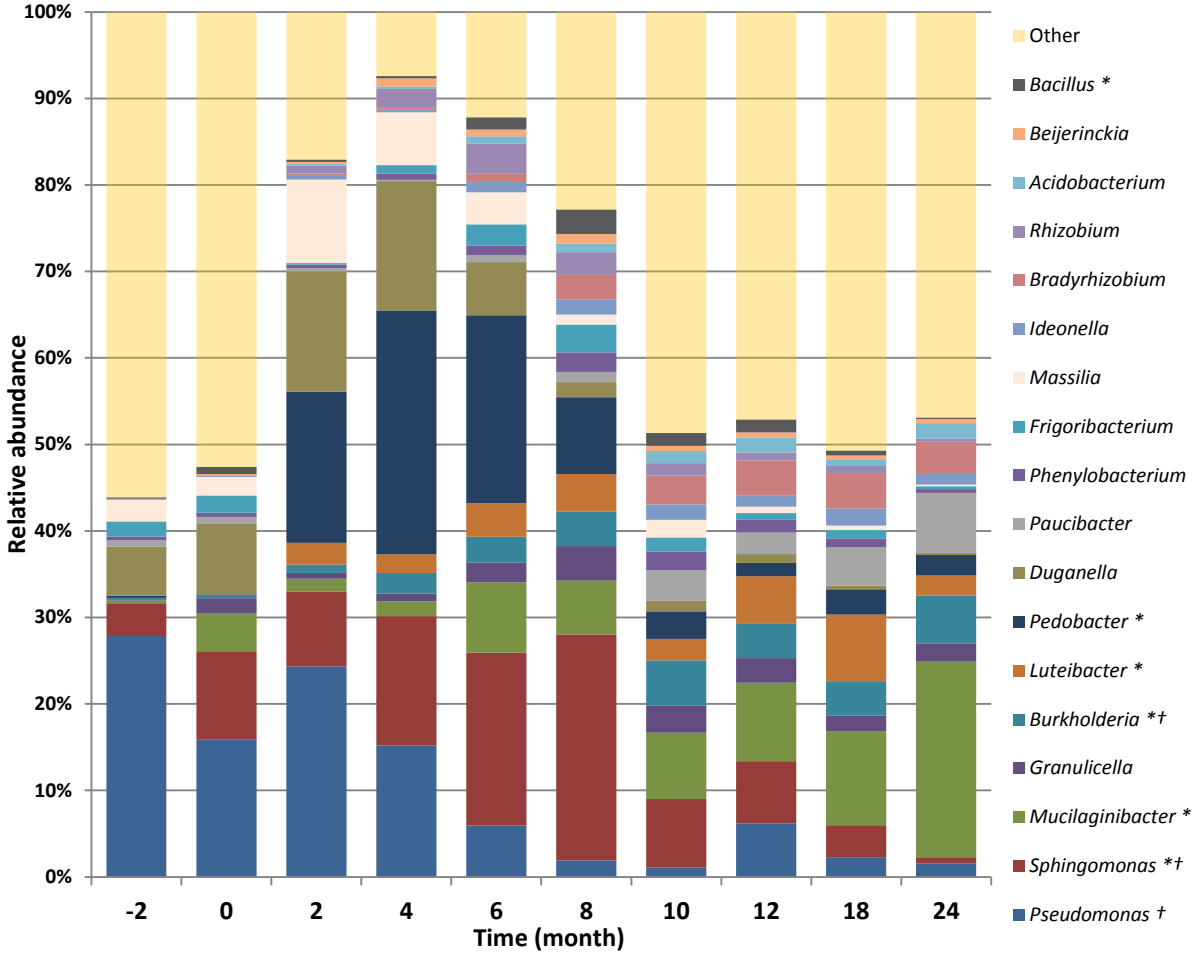


Fig. 9: Relative abundaces of bacterial genera on *Q. petraea* leaves and litter. Displayed genera showed abundances ≥ 0.5% in at least 20 samples from the whole set of samples. Taxa with lower abundances and unidentified OTUs are labeled as "Other". The data represent means of three or four replicate litterbags. * stands for genera with known ability to degrade cellulose, † indicates genera putatively involved in lignin modification. See Table S1 and S2 in the Appendix for the exact abundances data.

Genera where cellullolytic isolates were identified previously, underwent changes in abundances that exhibited two patterns. *Pedobacter* and *Sphingomonas* peaked in the middle phase of decomposition (28.2% in month 4 and 26.1% in month 8, respectively; Fig. 10). Others as *Mucilaginibacter*, *Burkholderia*, *Flavobacterium*, *Variovorax* and *Methylobacterium* showed gradual increase as decomposition proceeded and reached their highest abundances in the month 24 (22.7%, 5.5%, 1.0%, 0.6%, 0.02%, respectively).

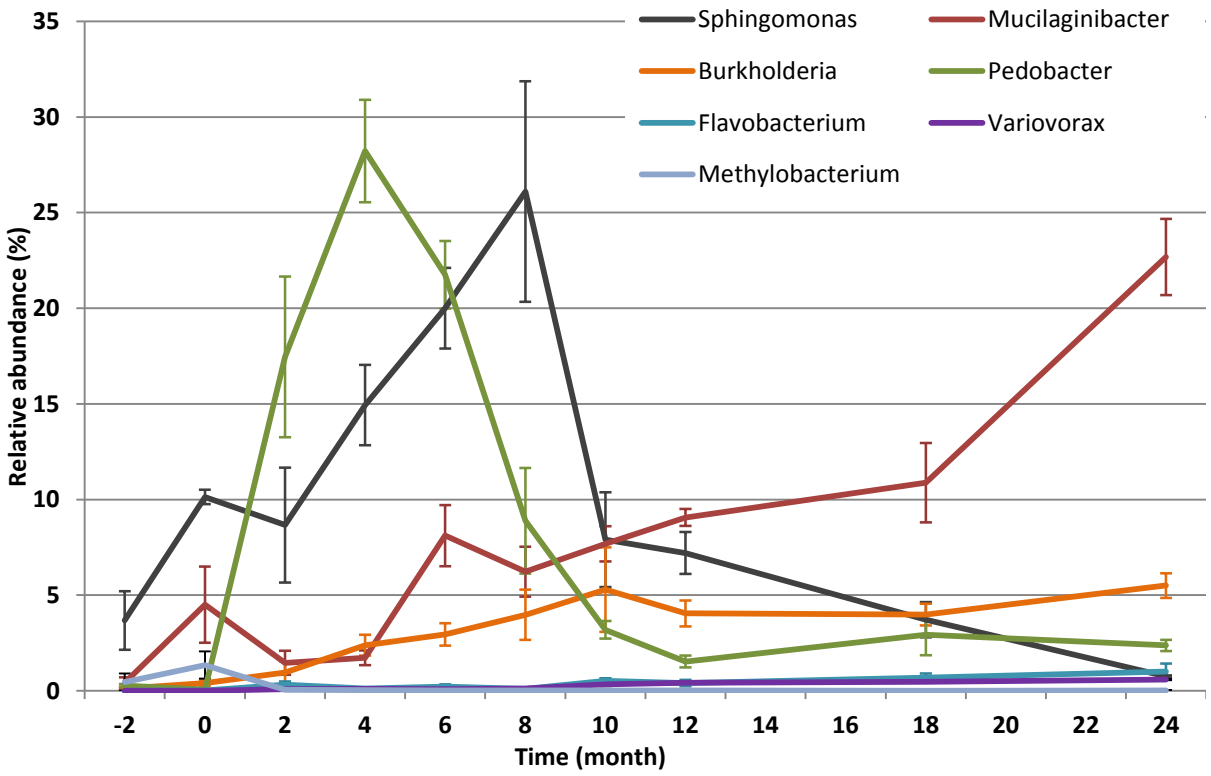


Fig. 10: Development of the relative abundances of prevalent bacterial cellulolytic genera on the leaves and litter of *Q. petraea*. Error bars represent standard errors.

Genera with abundance at least 1% in the month 2 were considered as early colonizers. This group comprised *Pseudomonas* (24.3%), *Pedobacter* (17.5%), *Duganella* (13.9%), *Massilia* (9.7%), *Sphingomonas* (8.7%), *Janthinobacterium* (5.8%), *Rahnella* (3.0%), *Luteibacter* (2.5%), *Acinetobacter* (2.3%) and *Mucilaginibacter* (1.5%). Sum of their relative abundances peaked in the months 2-4 and then markedly decreased towards low percentage in later decomposition

(Fig. 11). Notably, only *Pseudomonas*, *Duganella* and *Acinetobacter* showed abundances over 1% also in the months -2 and 0.

Similarly but from opposite side – genera with abundance at least 1% in the month 24 were considered as late saprotrophs. This group encompassed a wider spectrum of taxa listed in the Table 2. Sum of abundances of bacteria dominating litter on month 24 (abundance ≥ 5%) clearly increased with time reaching its maximum in the last month (Fig. 12). When considering taxa with 1% threshold, lower and flat peak of abundances was observed also in the months 2-6. It was a consequence of the presence of *Pseudomonas*, *Pedobacter* and also *Mucilaginibacter* and *Luteibacter* in the group of late saprotrophs as well as early colonizers.

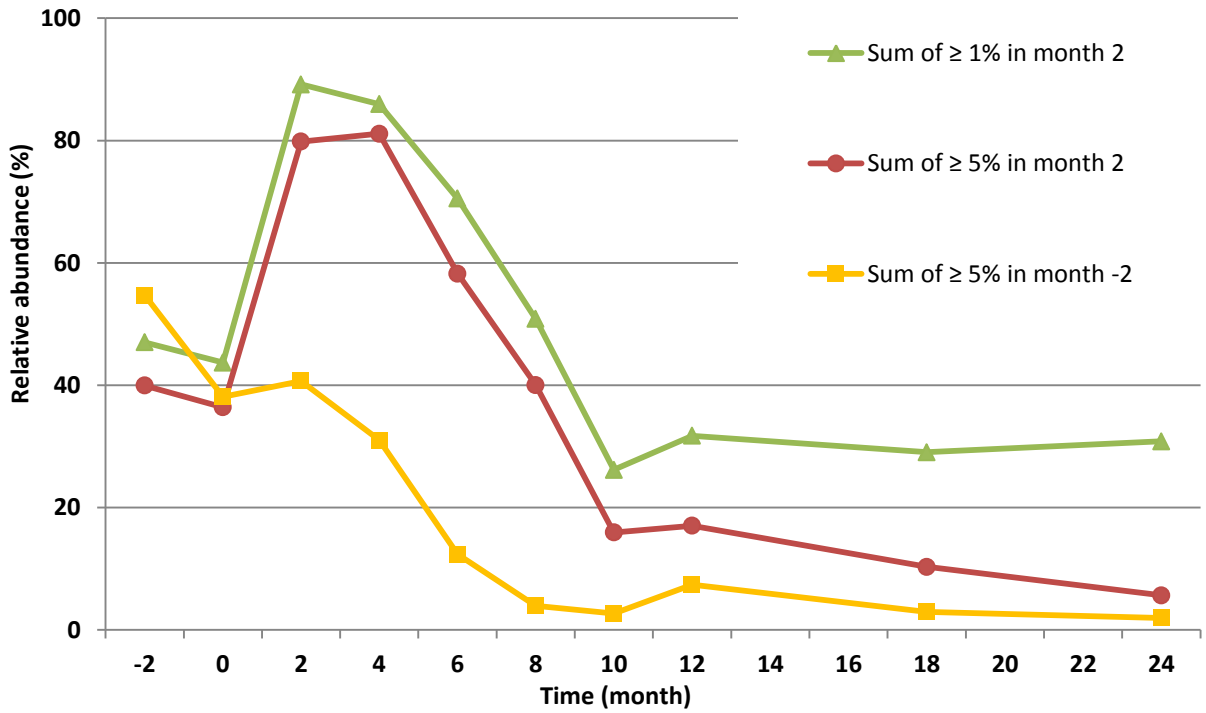


Fig. 11: Persistence of phyllosphere-associated and early-colonizer bacteria on *Q. petraea* leaves and litter. Data represent the mean sums of relative abundances for dominant genera with abundance ≥ 5% in the month -2 (phyllosphere, orange squares), genera with abundance ≥ 1% in the month 2 (green triangles) and genera with abundance ≥ 5% in the month 2 (red circles).

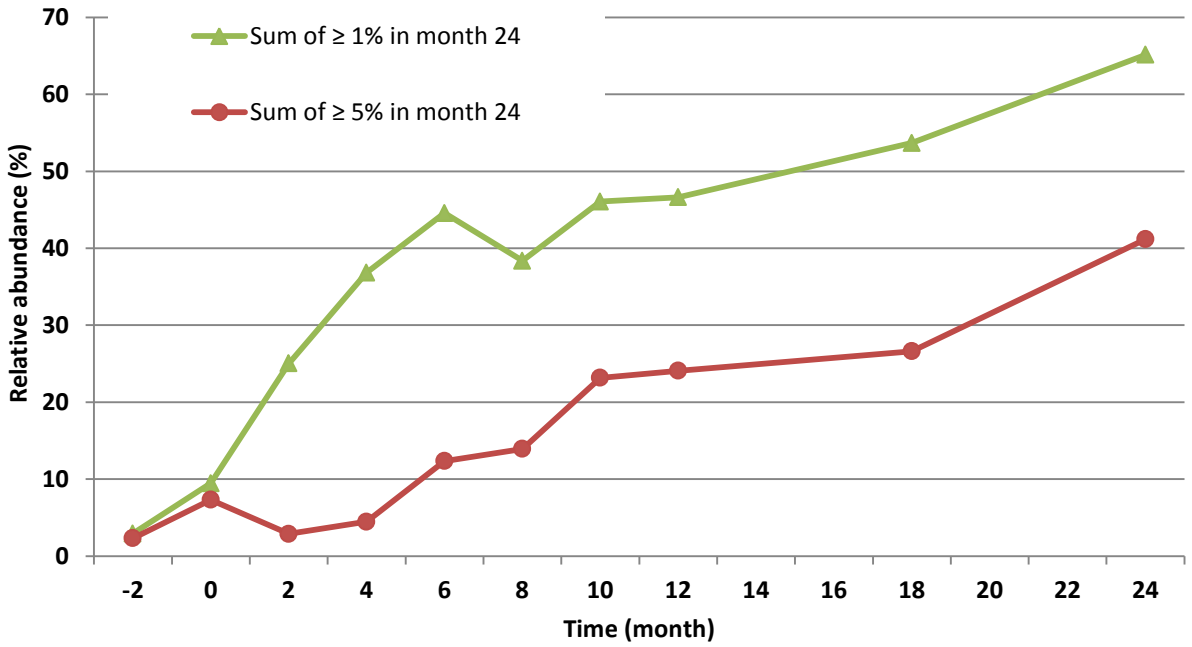


Fig. 12: Abundances of bacteria identified as late decomposers on *Q. petraea* leaves and litter. Data represent the mean sums of relative abundances for genera with abundance ≥ 1% (green triangles) and genera with abundance ≥ 5% (red circles) in month 24.

	Relative abundance (%) ± SE	
Genus	**Month 2**	**Month 24**
Mucilaginibacter	1.5 ± 0.5	22.7 ± 1.7
Paucibacter	0.4 ± 0.2	7.0 ± 0.4
unclass. genus	0.1 ± 0.0	6.0 ± 0.9
Burkholderia	1.0 ± 0.5	5.5 ± 0.6
Bradyrhizobium	0.2 ± 0.1	3.7 ± 0.8
Pedobacter	17.5 ± 3.4	2.4 ± 0.3
Luteibacter	2.5 ± 0.3	2.3 ± 0.2
Chthoniobacter	0.0 ± 0.0	2.3 ± 0.2
Chitinophaga	0.1 ± 0.1	2.2 ± 0.3
Mycobacterium	0.0 ± 0.0	2.1 ± 0.2
Granulicella	0.7 ± 0.4	2.0 ± 0.2
Acidobacterium	0.2 ± 0.1	1.8 ± 0.2
Pseudomonas	24.3 ± 7.3	1.5 ± 0.2
Ferruginibacter	0.1 ± 0.0	1.5 ± 0.2
Prosthecobacter	0.0 ± 0.0	1.3 ± 0.1
Ideonella	0.5 ± 0.1	1.2 ± 0.2
Flavobacterium	0.3 ± 0.1	1.0 ± 0.4

Table 2: Mean relative abundances and standard errors for genera with an abundance ≥ 1% in month 24. Values from month 2 are displayed for comparison.

5.1.2 Diversity indices

Predicted bacterial species richness on *Q. petraea* leaves and litter increased with time. The Chao-1 estimate showed the lowest value on months 0 and 2 (Fig. 13; month -2 was omitted because of low number of sequences insufficient for resampling to 1 500 sequences). According to Chao-1, number of species increased with time and reached the highest values during late decomposition. The months 10, 12 and 24 had significantly higher number of species than months 0-8.

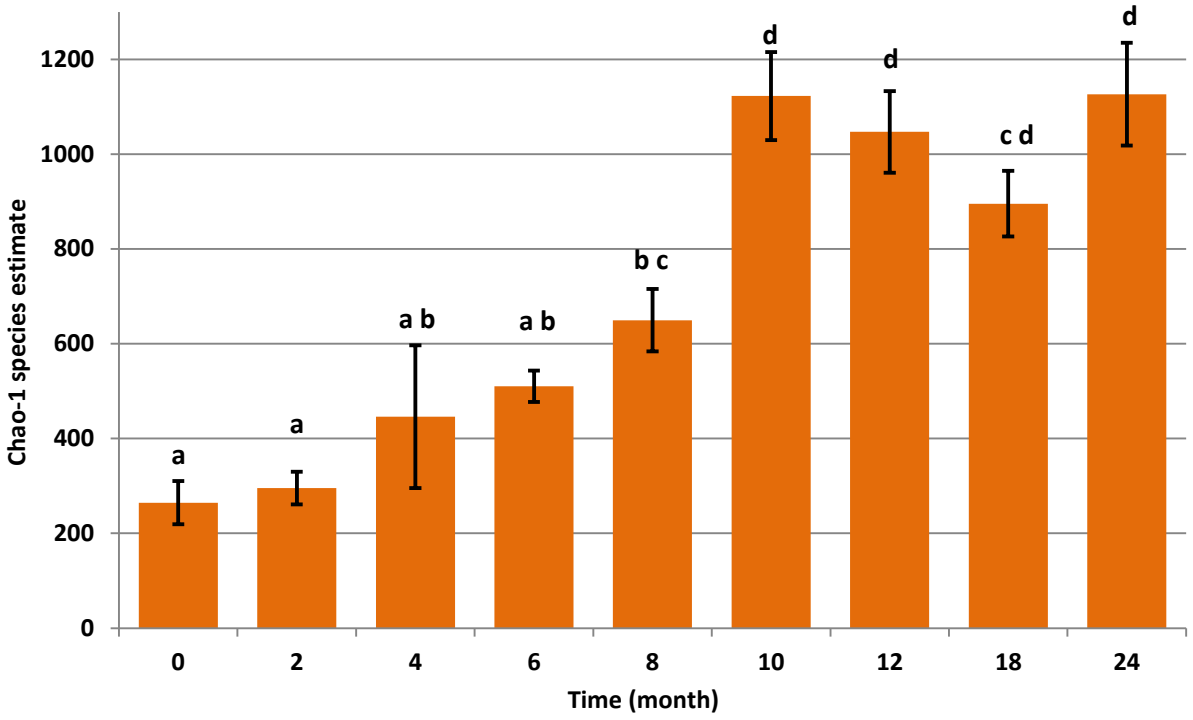


Fig. 13: Bacterial species richness on *Q. petraea* leaves and litter. Chao-1 estimates of total number of species for each month. The data represent means and standard errors of three or four replicate samples, subsampled at 1 500 sequences per sample. Statistically significant differences are indicated by different letters.

Values of Shannon-Wiener Diversity Index and Evenness provided similar results (Fig. 14). However, in this case, months 2 and 4 showed significantly the lowest diversity and evenness. The months 0, 6 and 8 were slightly more diversified. And late decomposition phase was characterized by significantly the richest and the most even community showing sharp transition between the months 8 and 10.

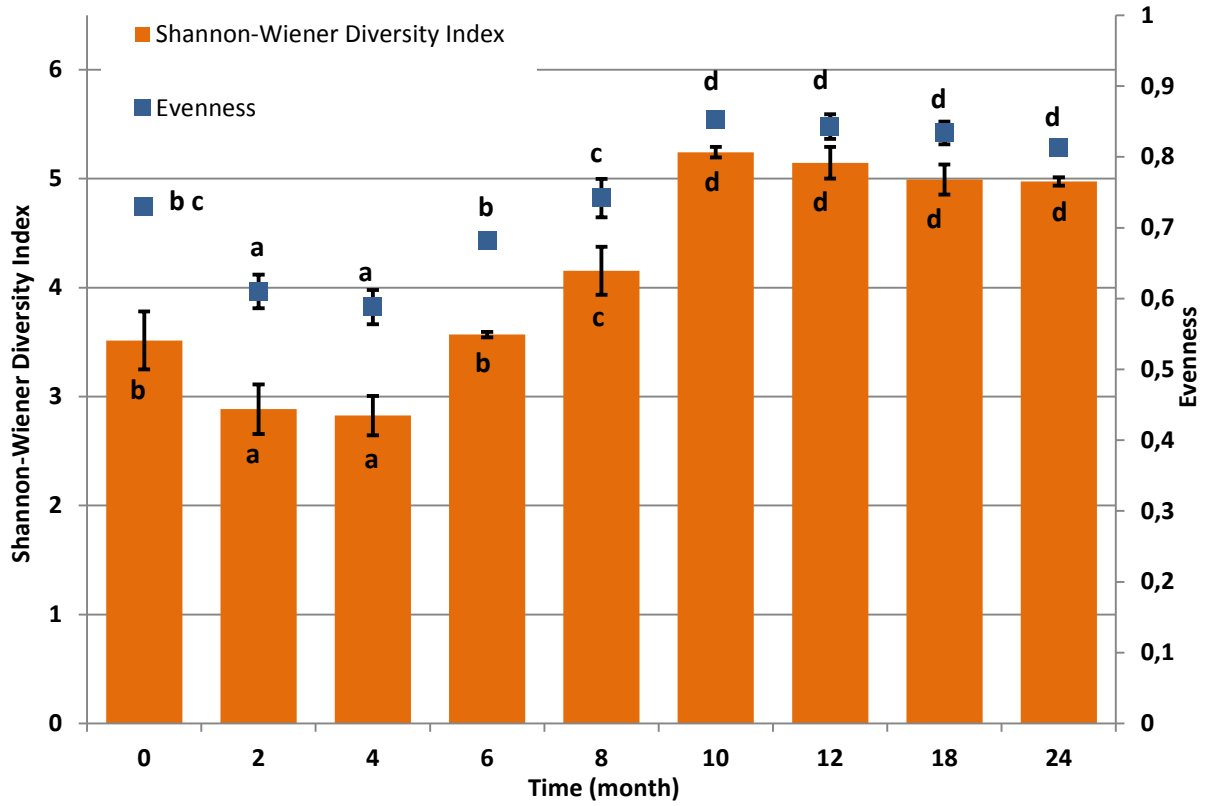


Fig. 14: Bacterial diversity and evenness on *Q. petraea* leaves and litter expressed as the Shannon-Wiener Diversity Index and Shannon Evenness. The data represent means and standard errors of three or four replicate samples, subsampled at 1 500 sequences per sample. Statistically significant differences are indicated by different letters.

5.1.3 Composition of *Q. petraea* leaves and litter and enzyme activity

Results of the litter dry mass loss, composition, values of microbial biomass and enzyme activities were published in the paper Šnajdr et al., (2011). Summary of the data follows (Fig. 15). After four months of decomposition, litter lost 16.4% of its dry mass, the mass lost after 12 months was 48.0% and at the end after 24 moths litter lost 68.0% of its initial mass. The mean water content was between 1.5 and 2.0 g H_2O g^{-1} dry mass. Drought period was recorded in the month 10 (0.2 g H_2O g^{-1}) and highest moisture content was recorded on month 24 (4.3 g H_2O g^{-1}). pH insignificantly varied between 4.3 and 4.7.

From total initial polysaccharides in leaf litter (month 0), hemicelluloses, cellulose and pectins represented 53%, 33% and 14%, respectively. 88% of initial cellulose and

79% of initial hemicelluloses mass was decomposed. Portion of decomposed lignin represented 66% of its initial mass. C:N ratio decreased from 49 to 22 after 12 months and than levelled off.

Relatively high activities of exocellulase, β-glucosidase and β-xylosidase in senescent leaves suggested initialized decomposition yet before abscission. Activities of these enzymes were high also after abscission and decreased during the months 4-6. Activities of endocellulase and endoxylanase showed high activity until month 12. Lignin-modifying laccase peaked on months 10 and 24 and manganese peroxidase on month 12. Month 12 was also characteristic by high activity of *N*-acetylglucosaminidase.

According to Mantel test, activities of endocellulase, exocellulase, β-glucosidase and laccase were significantly correlated with the composition of bacterial communities during decomposition.

PCA analysis of the abundances of bacterial genera was combined with the data on enzyme activities. The amount of biomass, pH, C and N content were used as covariables (Fig. 16). The plot indicates separation of samples from different months that are coded to visualize successional development. Their clustering into three groups is noticeable.

First two principal components explained 51.6% and 14.8% of the total variability. Months are sorted according to successional course and their positions allow to assess importance of displayed variables to particular phase of decomposition. The fungal:bacterial biomass ratio was pronounced during months -2 and 0. The highest activities of cellulases and hemicellulases (exocellulase, β-glucosidase, endocellulase and endoxylanase) were observed in the middle phase of decomposition. Bacterial biomass expressed as the PLFA content increased with time. In the cluster of months 10-24, laccase and Mn-peroxidase – enzymes for the degradation of non-cellulolytic recalcitrant compounds, were pronounced. Position of selected bacterial genera matched their dynamics described in the upper paragraphs (Fig. 16).

ANOSIM based on a Bray-Curtis similarity matrix of either all OTUs or on the set of the most abundant ones did not show significant differences between communities from different months of decomposition, likely due to the limited number of samples per timepoint (data not shown).

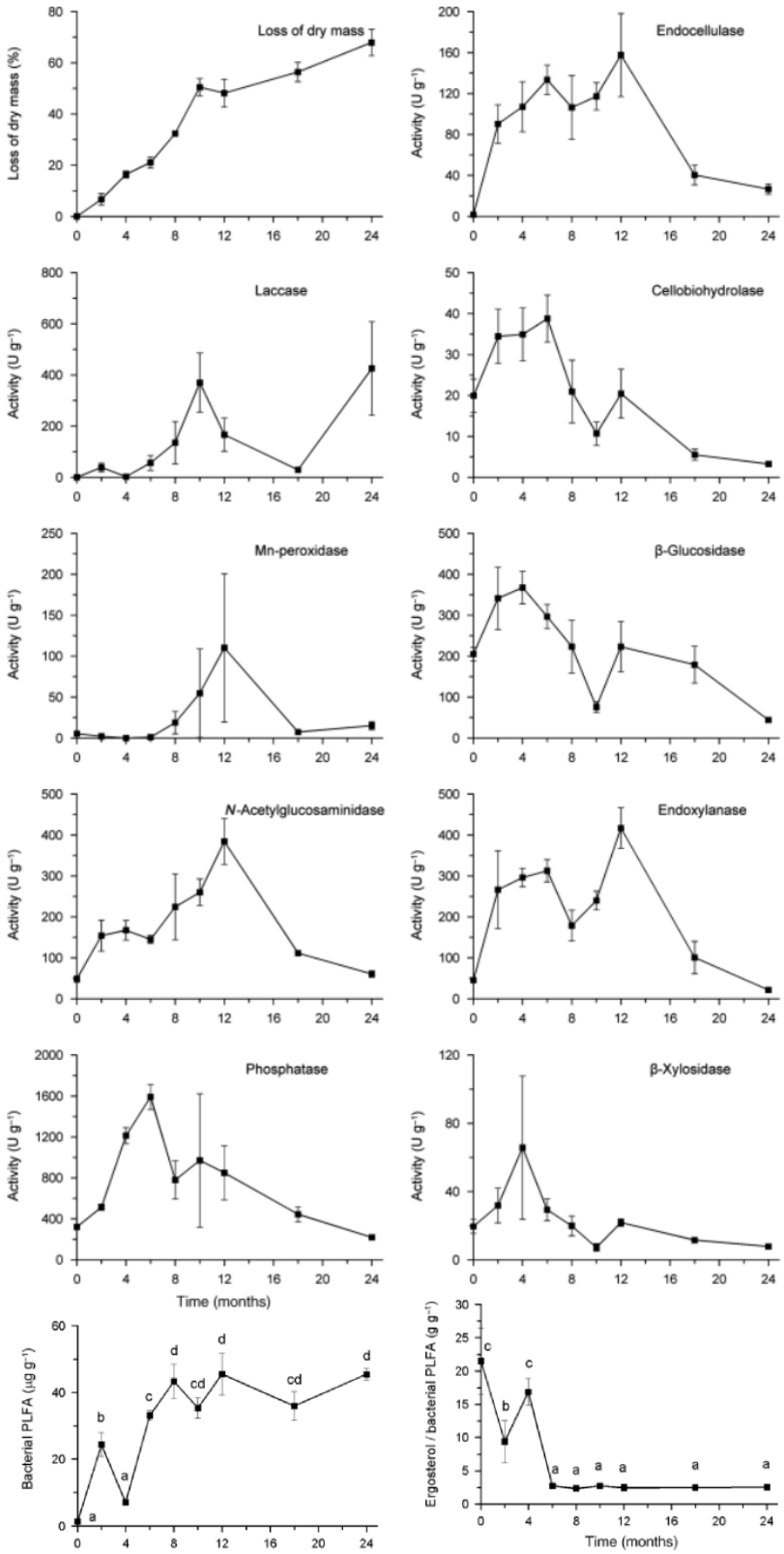


Fig. 15: Activities of extracellular enzymes, loss of dry mass, bacterial biomass and fungal:bacterial biomass ratio during two years of *Q. petraea* litter decomposition. The data represent means and standard errors of four replicate samples. Statistically significant differences are indicated by different letters. (data from Šnajdr et al., (2011)

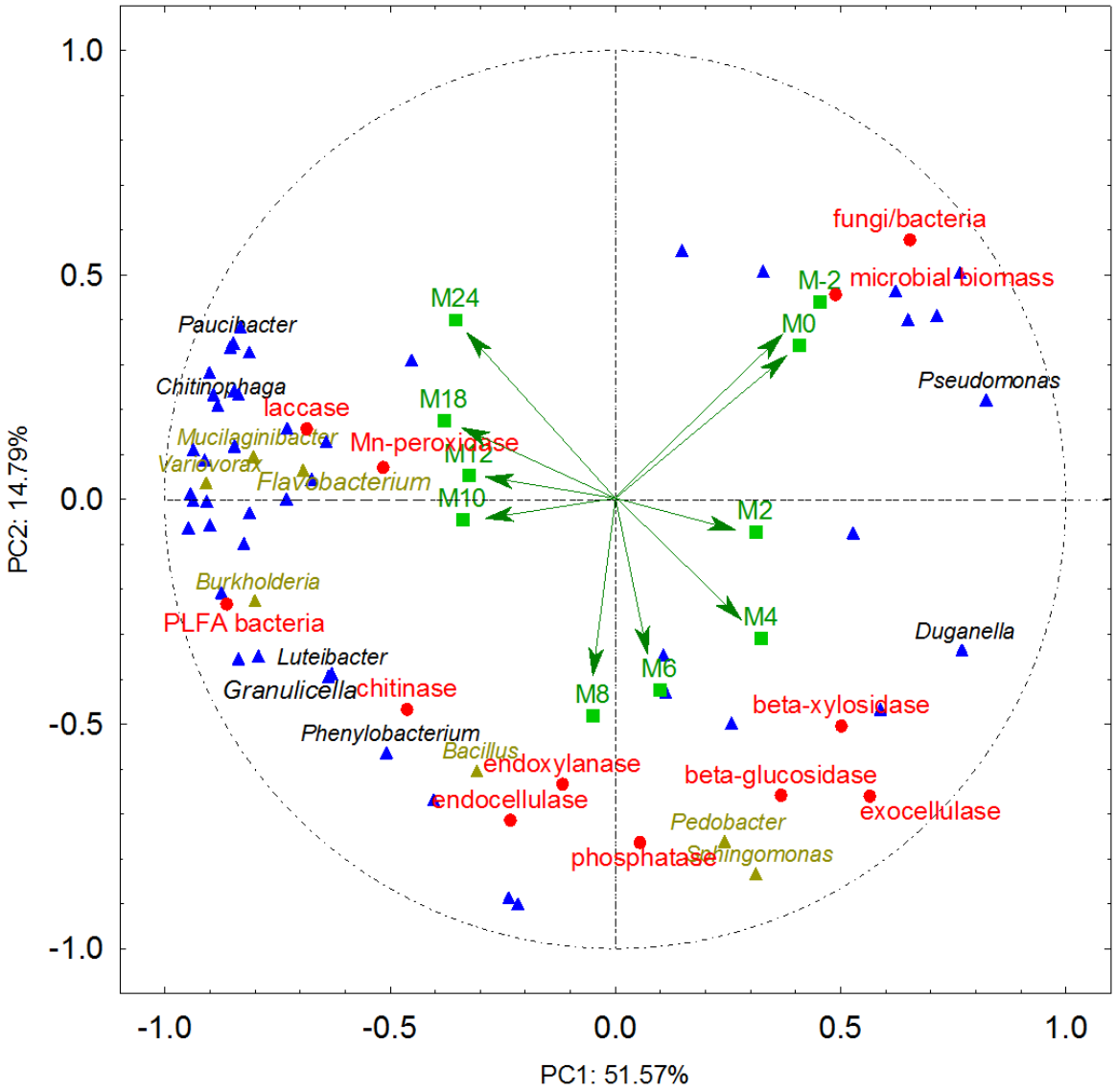


Fig. 16: PCA of the bacterial community composition on *Q. petraea* leaves and litter based on the abundances of 54 bacterial genera (abundances ≥ 0.5% in at leat 5 samples), values of enzyme activities and biomass (two latter factors displayed by red circles). The most abundant genera (blue triangles) and selected genera known for their cellulolytic activity (brown triangles) are titled. The importance of successional course presented by different months from M-2 to M24 (green squares) for ordination of factors is evident. Spots for enzyme activities together with genera are sorted according to the phase of decomposition in which they are important.

5.2 Bacterial communities during initial decomposition of deadwood

5.2.1 Composition of community associated with deadwood of different species and size

Sequencing and quality filtering delivered 1 046 509 and 432 193 sequences from the years 2012 (in the text also as the year 1) and 2013 (year 2), respectively. Sequences were clustered into 22 200 OTUs at the 97% similarity threshold (Table 3).

		Year 1 (2012)					Year 2 (2013)			
	total 2012	CWD/B	CWD/T	FWD/B	FWD/T	total 2013	CWD/B	CWD/T	FWD/B	FWD/T
number of samples	124	31	31	31	31	123	31	30	31	31
number of sequences	1 046 509	175 713 (16.8%)	302 292 (28.9%)	272 904 (26.1%)	295 600 (28.2%)	432 193	112 022 (25.9%)	135 650 (31.4%)	95 615 (22.1%)	88 906 (20.6%)
mean sequences/sample	8 440	5 668	9 751	8 803	9 535	3 514	3 614	4 522	3 084	2 868
median sequences/sample	9 713	6 369	9 769	9 720	9 704	3 327	3 385	4 534	2 736	2 842
number of OTUs	22 200	4 362 (19.6%)	5 427 (24.4%)	6 542 (29.5%)	7 914 (35.6%)	22200	2 581 (11.6%)	3 364 (15.2%)	3 096 (13.9%)	3 723 (16.8%)
mean OTUs/sample	548	365	479	578	770	415	337	468	393	463
median OTUs/sample	542	386	478	562	741	399	307	470	392	445
relative abundance of top* OTUs	59.3%	52.5%	69.6%	61.8%	53.5%	50.0%	50.9%	49.0%	54.9%	45.1%

Table 3: Overview of sequencing results of bacterial communities associated with decomposing deadwood during two years of deadwood decomposition. * top OTUs had relative abundances ≥ 0.5% in at least 30 samples in each year; these OTUs are listed in the Table S3 and S5 in the Appendix.

Alphaproteobacteria, *Gammaproteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Betaproteobacteria* represented the most abundant taxa during two years of decomposition, irrespective of sample type (Fig. 17). Abundances ≥ 0.5% of these taxa were recorded in all samples from particular year. The phylum *Firmicutes* showed abundances over this threshold in all samples only in year 2 although nearly all samples (122) contained at least 0.5% of *Firmicutes* in year 1. Abundance of *Firmicutes* showed significantly lower values in FWD when considering both years together. The phylum *Acidobacteria* showed lesser abundances but it was still present in a majority of the samples from both years. In year 1, *Acidobacteria* showed

significantly higher abundance in fir FWD. For the phylum *Deltaproteobacteria*, slightly higher insignificant abundance was observed in year 2. The phylum *Verrucomicrobia* showed significant increase in abundances in year 2 in all sample types except beech CWD.

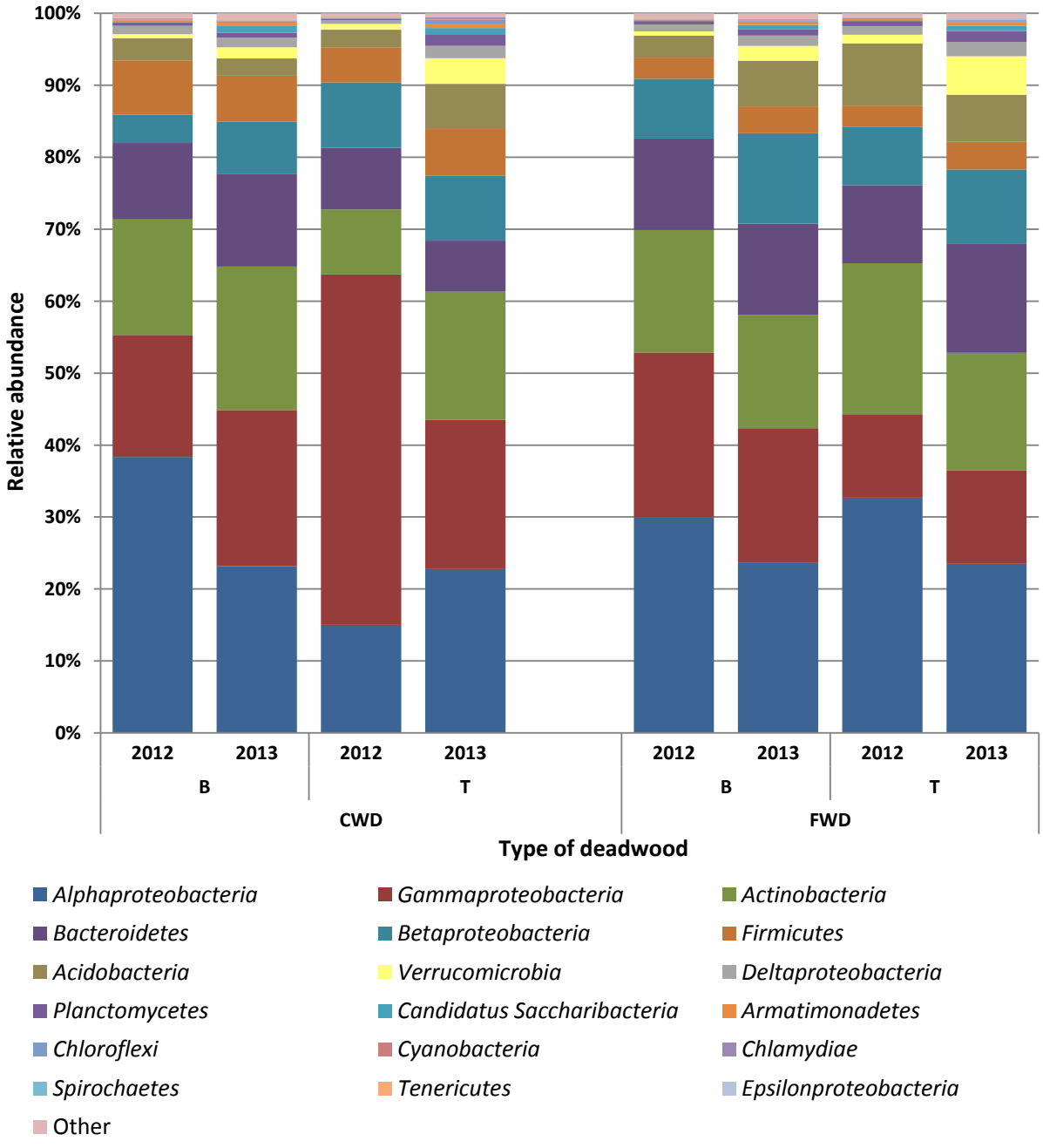


Fig. 17: Relative abundaces of bacterial phyla (or classes in *Proteobacteria*) in deadwood experiment with coarse woody debris (CWD) and fine woody debris (FWD) of beech (B) and fir (T) from years 2012 and 2013. Taxa with relative abundances ≥ 0.5% in at least 1 out of 124/123 samples are displayed. The data represent means of replicates for each type of deadwood. Less abundant phyla and unidentified OTUs are indicated as "Other".

Previously recognized cellulolytic genera *Sphingomas*, *Mucilaginibacter, Burkholderia* and *Pedobacter* were among the most abundant as well as the members of the genus *Pseudomonas* whose members were previously reported as potentially lignin-modifying (Fig. 18). Lignin-modifying activity is described also for genera *Sphingomas* and *Burkholderia*. Other abundant genera were *Bradyrhizobium* and *Frigoribacterium*, although their potential capability for plant biomass deconstruction is not described. Two last mentioned genera displayed significantly higher abundances in beech FWD in year 1 while in year 2 higher abundances in fir CWD and beech CWD were recorded for *Bradyrhizobium* and *Frigoribacterium*, respectively. *Sphingomonas* as the most prevalent genus did not show differences between deadwood types while *Mucilaginibacter* and *Burkholderia* were significantly more abundant in FWD of beech and fir from both years. *Pedobacter* was less abundant in CWD of fir in both years. On the other hand, abundance of *Pseudomonas* was significantly higher in fir CWD in year 1. In the year 2, its abundances did not differ among deadwood types. Another cellulolytic genus *Dyella* was abundant during year 1 only in beech FWD while in the second year it showed high abundances in FWD of both tree species. *Cellulomonas* was abundant in beech CWD in both years while cellulolytic *Luteibacter* was abundant in beech FWD in both years.

High abundance in fir CWD was recorded for genus *Rahnella*. This genus showed distinctly high prevalence in fir CWD in year 1 in comparison with all other types of deadwood. The large OTU C000000 assigned to the genus *Rahnella* (Fig. 19) was also among important indicator species for fir CWD (Table S7 in the Appendix). *Methylosinus* was another genus with higher abundance in beech CWD in year 1. Although *Methylosinus* was abundant in beech CWD, the OTU C000027 assigned to this genus was an indicator species in fir FWD.

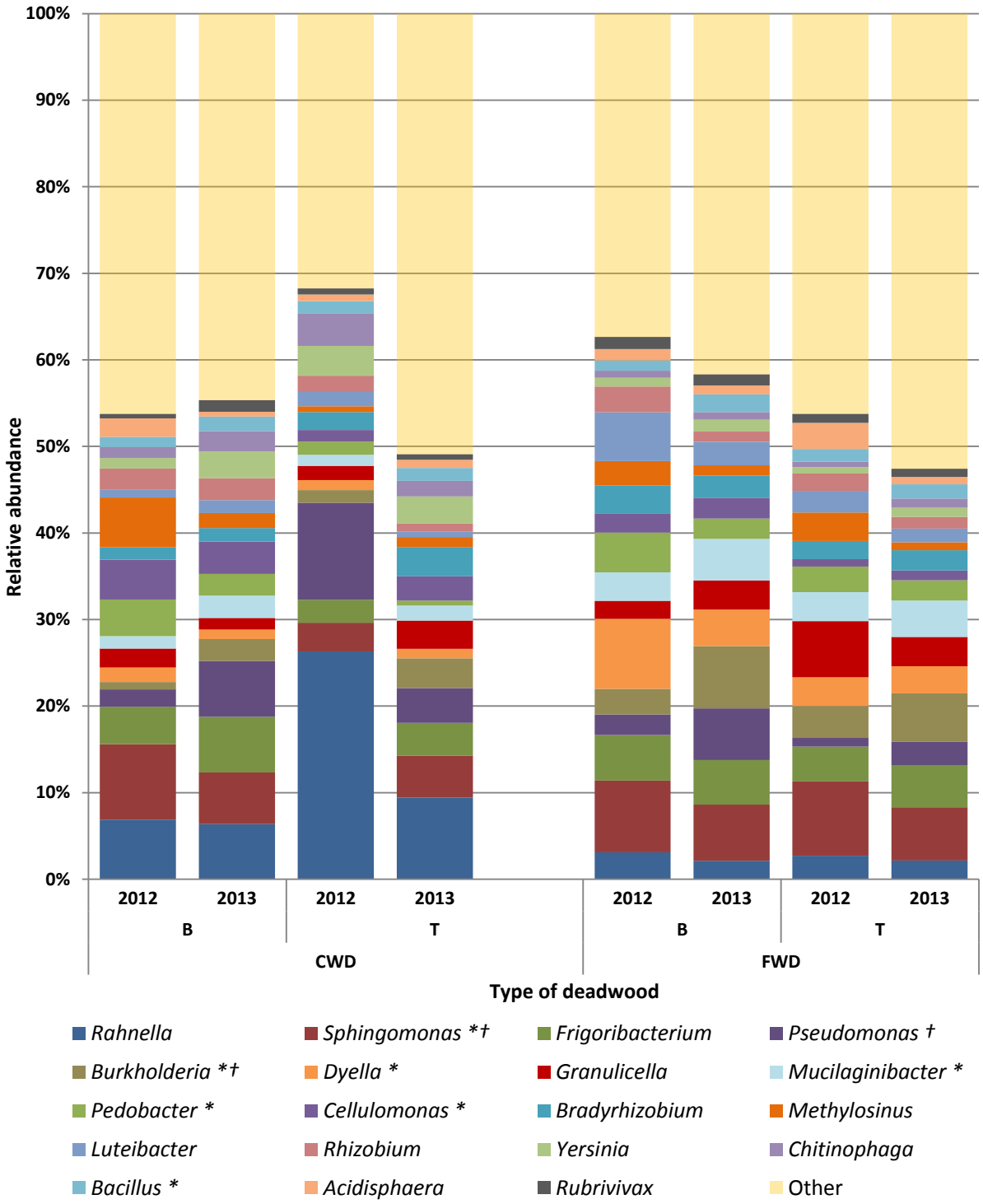
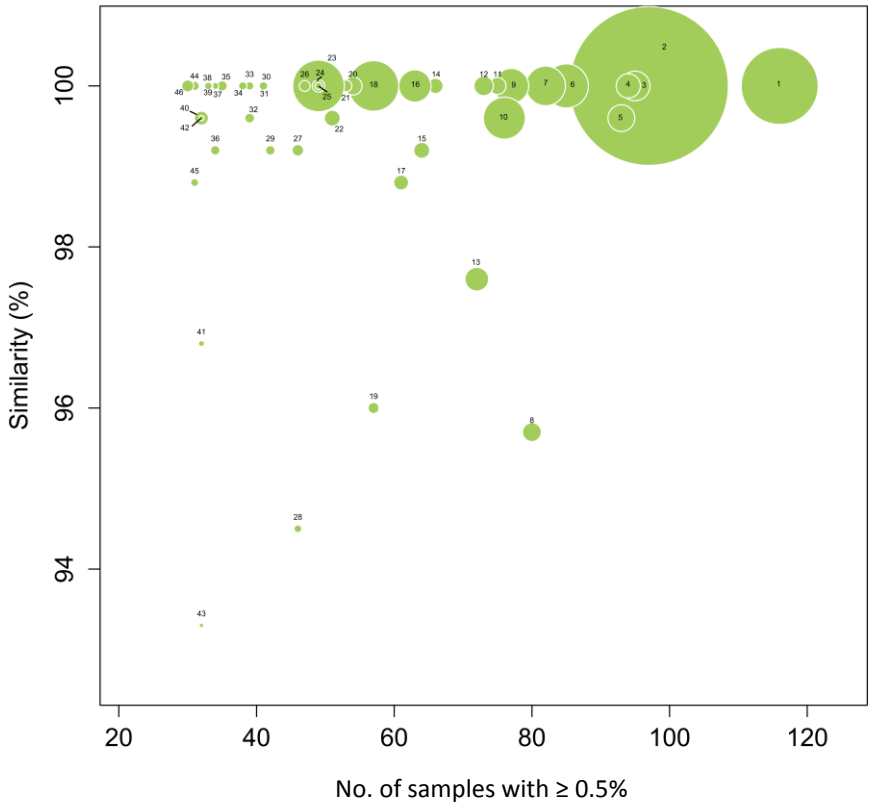
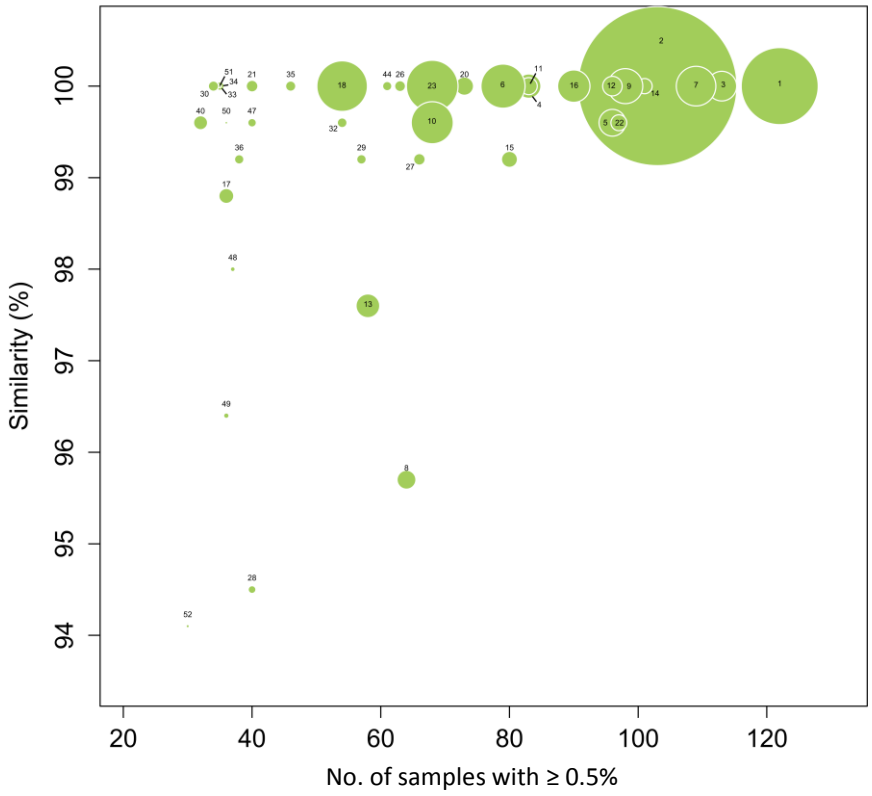


Fig. 18: Relative abundaces of bacterial genera (or classes in *Proteobacteria*) in deadwood experiment with coarse woody debris (CWD) and fine woody debris (FWD) of beech (B) and fir (T) from years 2012 and 2013. The data represent means of replicates for each type of deadwood. Displayed genera showed abundances ≥ 0.5% in at least half of the samples from the both years. Genera with lower abundances and unidentified OTUs are indicated as "Other". * stands for genera with known ability to degrade cellulose, † indicates genera putatively involved in lignin modification. See Table S4 and S6 in the Appendix for the table of values.

A


Similarity (%)
100
98
96
94
20
40
60
80
100
120
No. of samples with ≥ 0.5%

B


Similarity (%)
100
99
98
97
96
95
94
20
40
60
80
100
120
No. of samples with ≥ 0.5%

C

Cluster	Best hit (accession number)	Bubble No.	Cluster	Best hit (accession number)	Bubble No.
C000001|n=66211	*Frigoribacterium* (JQ977229)	1	C000026|n=9914	*Jatrophihabitans* (JQ346802)	27
C000000|n=136621	*Rahnella* (KC351183)	2	C000042|n=6654	*Acidiphilium* (KC924950)	28
C000009|n=25490	*Bradyrhizobium* (JN221510)	3	C000033|n=8181	*Amnibacterium* (KC251736)	29
C000011|n=20894	*Rhizobium* (KC853173)	4	C000029|n=8775	*Duganella* (JF778667)	30
C000010|n=23035	*Sphingomonas* (KC735149)	5	C000037|n=7350	*Sphingomonas* (EF558729)	31
C000004|n=37902	*Luteibacter* (AB627008)	6	C000031|n=8363	*Acidisoma* (AM947652)	32
C000006|n=34052	*Burkholderia* (KF031512)	7	C000039|n=7172	*Beijerinckia* (FR874230)	33
C000014|n=16203	*Methylosinus* (AJ458477)	8	C000040|n=7140	*Ideonella* (KC355350)	34
C000007|n=30061	*Cellulomonas* (KC337106)	9	C000030|n=8774	*Burkholderia* (HE814630)	35
C000005|n=36143	*Pedobacter* (HE814666)	10	C000034|n=8058	*Sphingomonas* (EF467848)	36
C000016|n=14664	*Rubrivivax* (AM086242)	11	C000046|n=6056	*Curtobacterium* (KC810833)	37
C000013|n=16791	*Bacillus* (JQ437583)	12	C000036|n=7650	*Rhizobium* (HF566319)	38
C000012|n=20383	*Sphingomonas* (EF467848)	13	C000043|n=6449	unclass. bacterium (EU476046)	39
C000019|n=13064	*Sphingomonas* (AB744218)	14	C000021|n=12009	*Undibacterium* (KC505152)	40
C000017|n=13726	*Granulicella* (KC924939)	15	C000050|n=5193	*Phenylobacterium* (JX949351)	41
C000008|n=27809	*Yersinia* (AJ011333)	16	C000054|n=4483	*Granulicella* (FR716684)	42
C000020|n=12811	*Acidisphaera* (AF376024)	17	C000061|n=3881	*Methylopila* (KC447360)	43
C000003|n=43440	*Dyella* (AB245367)	18	C000035|n=7956	*Mycobacterium* (FR693307)	44
C000027|n=9577	*Methylosinus* (AJ458477)	19	C000041|n=6749	unclass. *Actinobacteria* (X68459)	45
C000015|n=15654	*Pseudomonas* (JN167957)	20	C000023|n=10317	*Stenotrophomonas* (KC790309)	46
C000025|n=10019	*Mucilaginibacter* (JX268541)	21	C000038|n=7335	*Gryllotalpicola* (JQ864374)	47
C000018|n=13650	*Mucilaginibacter* (EU423302)	22	C000059|n=4131	*Acidobacterium* (AM086241)	48
C000002|n=44396	*Pseudomonas* (HF913576)	23	C000052|n=4673	*Gluconacetobacter* (AB627120)	49
C000022|n=10562	*Erwinia* (KC677746)	24	C000097|n=2322	unclass. *Flavobacteria* (AF491884)	50
C000044|n=6126	*Novosphingobium* (KF145130)	25	C000088|n=2634	*Dokdonella* (AB245362)	51
C000028|n=9327	*Bradyrhizobium* (HQ694740)	26	C000085|n=2750	*Rhizomicrobium* (AB081581)	52

Fig. 19: The most abundant OTUs identified in deadwood experiment. OTUs displayed in the bubble graphs had abundances ≥ 0.5% in at least 30 samples in year 1 (**A**) and year 2 (**B**). Area of green bubbles is proportional to the number of sequences which were clustered into OTU. Position of OTUs on *x* axis indicates number of samples from particular year over abundance threshold 0.5%. Position on *y* axis indicates similarity value (%) of the OTU as compared by BLASTn against NCBI database. See table **C** for coding of OTUs. The same OTUs with additional values are listed in Tables S3 and S5 in the Appendix.

PCA analysis was done for two years separately on the square root transformed data of relative sequence abundances for all genera with abundances ≥ 0.5% in at least three samples. Enzyme activities, pH, C and N content of deadwood, tree species and deadwood size were used as supplementary variables.

First two principal components explained 18.53% and 9.78% of the total variability (Fig. 20). Deadwood size was the key driving factor of community composition in the first year. On the other hand, tree species had only minor role in this very initial phase of decomposition. pH of the sample was identified as another

factor strongly influencing bacteria. This is in accordance with repeatedly confirmed strong response and lability of bacteria to changing pH. Distributions of enzymes as measured by their activity did not provide any clear pattern. It suggests that during initial decomposition of wood targeted by this experiment, enzyme activities can not be differentiated into subsequent phases as has been shown for decomposition of more labile leaf litter (Fig. 16).

In the PCA of the data from year 2, the first two principal components explained 12.9% and 9.3% of the total variability (Fig. 21). Tree species and deadwood diameter were equally important drivers of community structure. Acidity or alkalinity of substrate was also a potentially important factor, moreover its importance slightly increased. So far, clear pattern in enzyme activities was not observed.

Based on ANOSIM it is possible to compare communities as a whole units and test whether they are similar or not. The higher R-value for particular combination of deadwood is, the more dissimilar are inhabiting communities. ANOSIM was performed for two years separately (Fig. 22). According to this analysis communities in beech CWD and FWD were more similar to each other while communities in fir CWD/FWD were more similar to communities from corresponding diameter of beech deadwood. This result was true for both years. All comparisons had significant p-values.

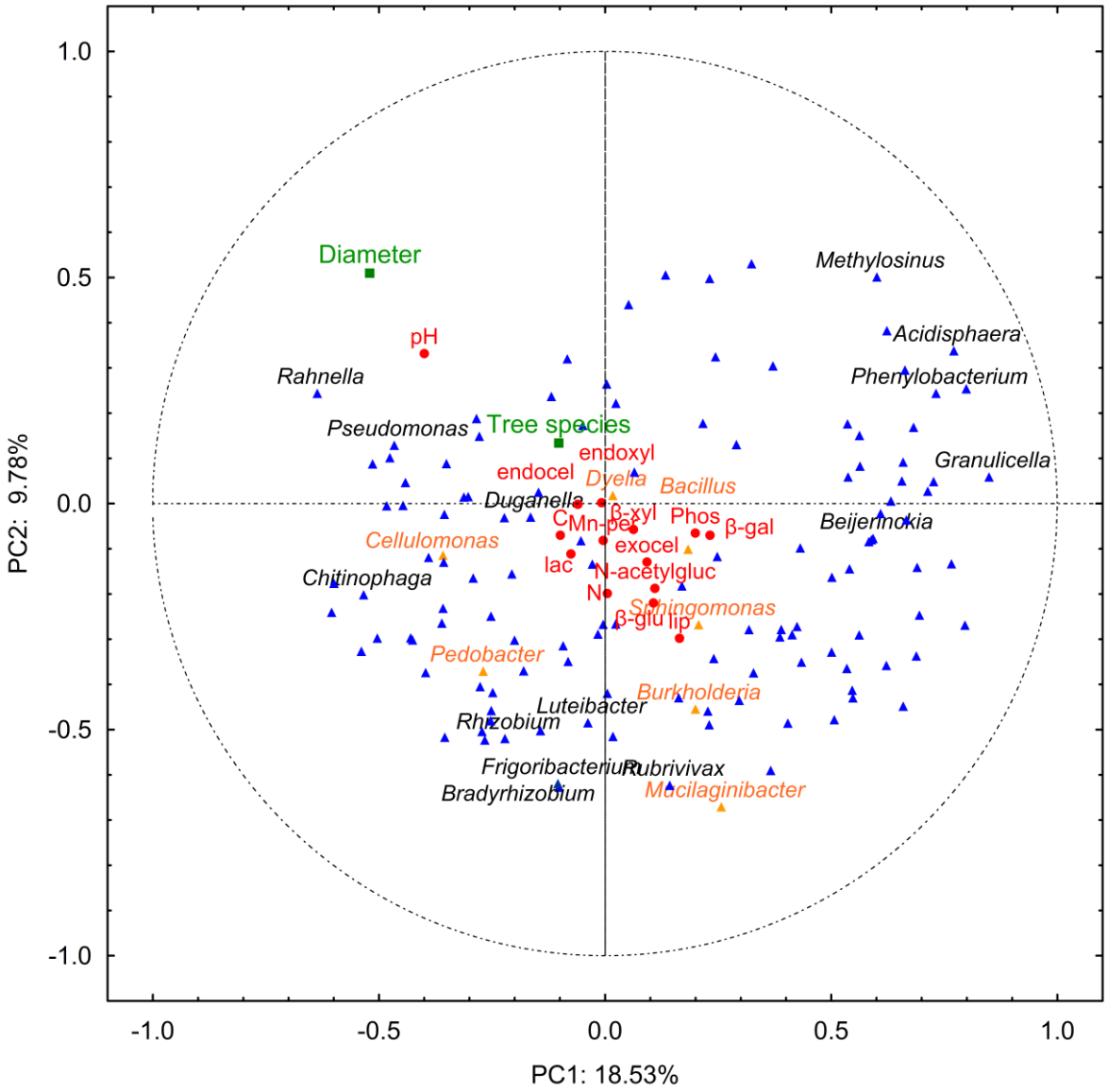


Fig. 20: PCA of the 130 most abundant genera from year 1 (with abundances ≥ 0.5% in at least three samples), values of enzyme activities, pH, C and N content. The most abundant genera and selected genera with cellulolytic activity (brown triangles) are titled. The least abundant titled cellulolytic genus *Dyella* had abundances ≥ 0.5% in 63 samples. Some species from genera *Sphingomonas*, *Pseudomonas* and *Burkholderia* are known also for lignin-modifying activity. Factors of tree species (beech/fir) and diameter of wood (CWD/FWD) are displayed as well (green squares). Square root of relative abundances of bacterial genera was used in PCA.

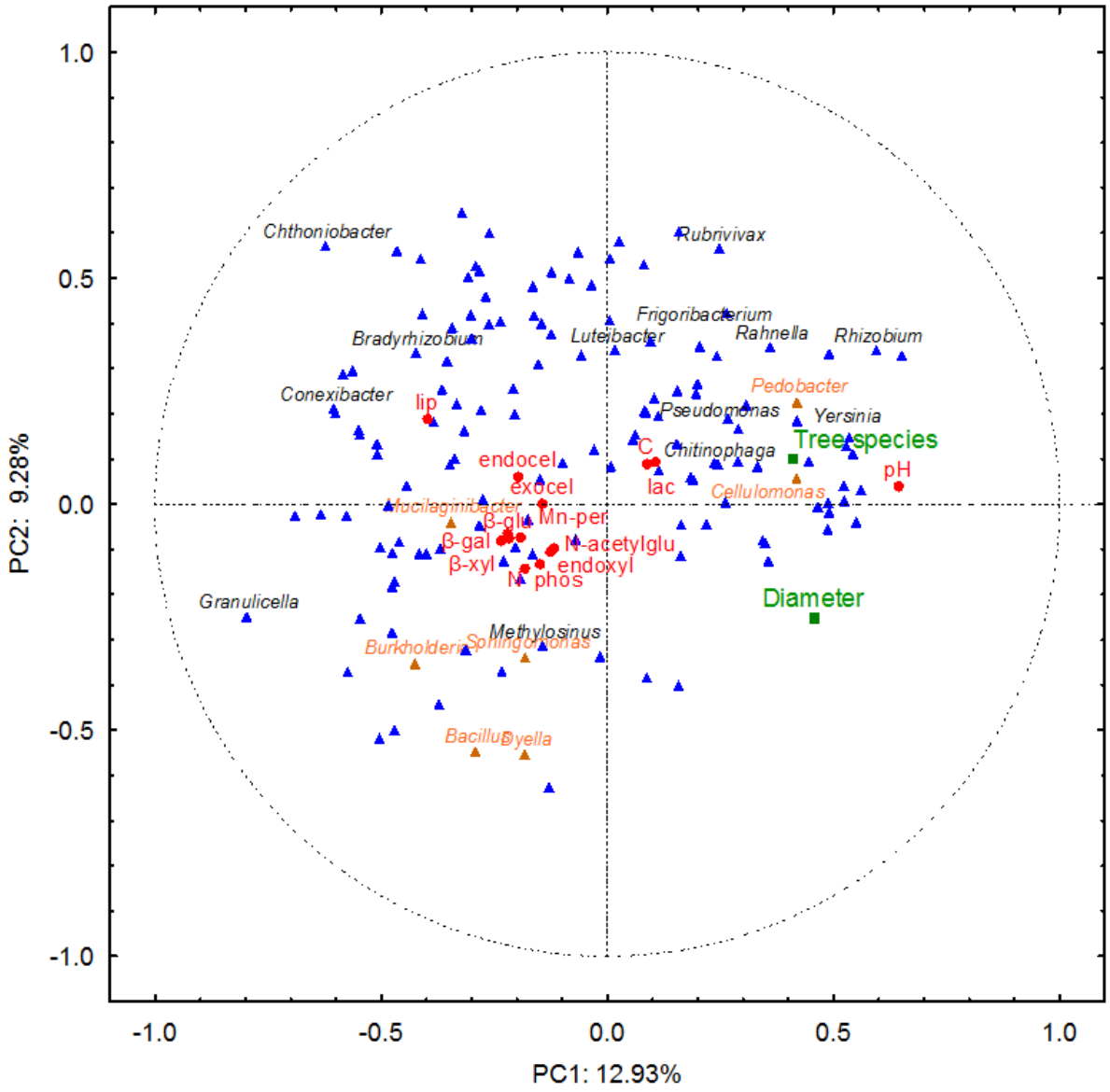


Fig. 21: PCA of the 145 most abundant genera from year 2 (with abundances ≥ 0.5% in at least three samples), values of enzyme activities, pH, C and N content. The most abundant genera and selected genera with cellulolytic activity (brown triangles) are titled. The least abundant titled cellulolytic genus *Dyella* had abundances ≥ 0.5% in 75 samples. After second year of decomposition factors of wood diameter (CWD/FWD) and tree species (beech/fir) were comparable in their influence of bacterial community composition (green squares). Square root of relative abundances of bacterial genera was used in PCA.

A

	CWD/B	FWD/B	CWD/T	FWD/T
CWD/B		0,2496	0,2645	0,3473
FWD/B	0,2089		0,3978	0,0958
CWD/T	0,4825	0,6062		0,3432
FWD/T	0,4266	0,3077	0,8242	

YEAR 1 / YEAR 2

B

	CWD/B	FWD/B	CWD/T	FWD/T
CWD/B		0,0006	0,0006	0,0006
FWD/B	0,0006		0,0006	0,0048
CWD/T	0,0006	0,0006		0,0006
FWD/T	0,0006	0,0006	0,0006	

YEAR 1 / YEAR 2

Fig. 22: Similarities of bacterial communities as function of deadwood type based on ANOSIM test. R-values (**A**) and corresponding p-values with Bonferroni correction (**B**) were derived from all OTUs and their Bray-Curtis similarity matrix (9 999 permutations). Note that year 1 and year 2 were analysed and coloured separately. The greener (year 1) and redder (year 2) cell with R-value is, than communities for particular combination of deadwood are more similar.

5.2.2 Diversity indices

The highest Chao-1 OTU richness predictor (at the sampling depth of 1 000 sequences per sample) was obtained for fir FWD in the first and second year (537 and 488 OTUs, respectively). It significantly differed from all other types of deadwood. In the year 1, fir CWD and beech FWD were not significantly different. As well in the year 2, CWD of both beech and fir were not significantly different showing the lowest estimates of the whole dataset (306 and 292 OTUs, respectively). Considering only one tree type of different diameter from single year there were significantly higher abundances counted for FWD in all cases. Interseasonal differences within one tree species and the same deadwood size were significant only in the case of fir CWD which showed higher OTUs estimate in year 1.

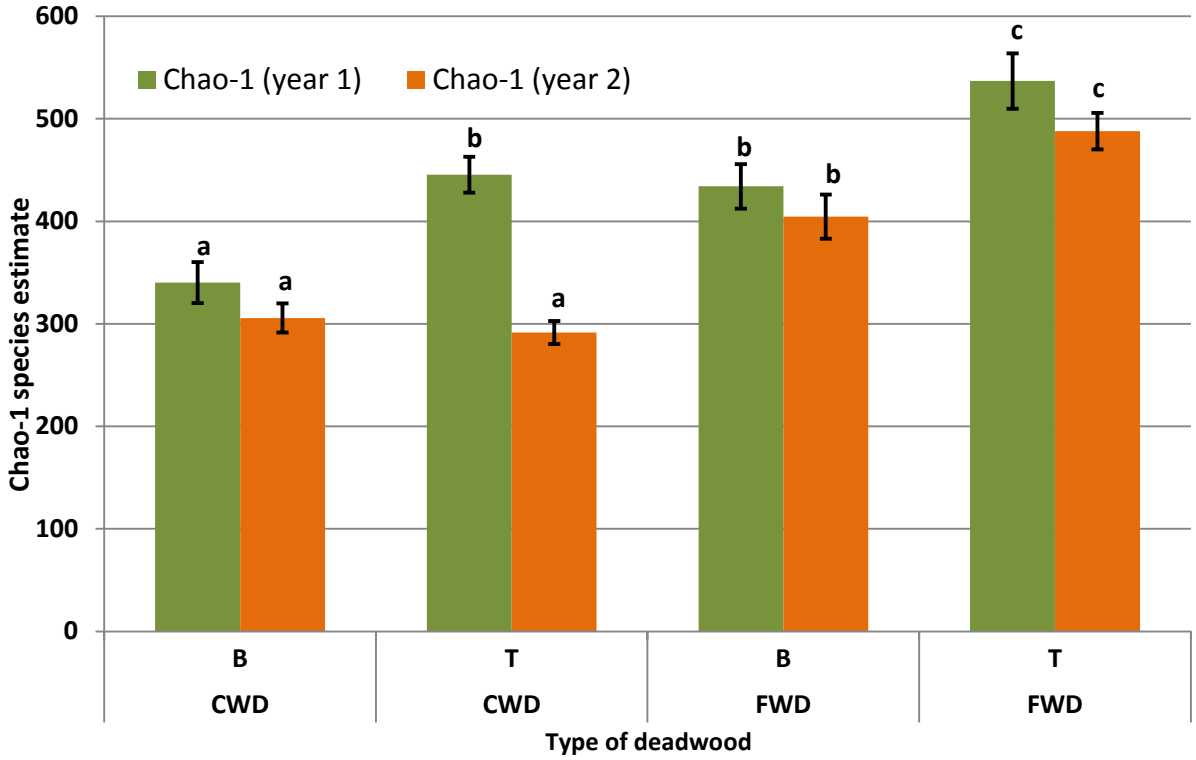


Fig. 23: Chao-1 estimate of total number of species for types of deadwood. Sequences from each sample in both years were randomly resampled to the number of 1 000. Standard errors are displayed, statistically significant differences are indicated by different letters (separately for two years).

Shannon-Wiener Diversity Index and Evenness were used for assessing diversity with same resampled sequences as for Chao-1 estimate. In year 1, fir CWD showed the lowest diversity and evenness while fir FWD showed significantly higher diversity than other deadwood types (Fig. 24). Further, rarefaction curves of fir CWD were not as steep as rarefaction curves of other deadwood types (data not shown). In other words, good sequencing depth unveiled low diversity in fir CWD and rich bacterial communities in fir FWD. In the year 1, FWD and CWD of beech did not significantly differ in their diversity.

Diversity and evenness of bacterial communities in the year 2 was more similar among treatments (Fig. 25). As in the year 1, fir FWD displayed the highest diversity. In fir CWD diversity was lower. Similarly in this year diversity in beech FWD was higher than in the beech CWD. In interseasonal comparison of corresponding deadwood types, fir CWD and beech FWD showed significant increase in diversity and evenness with time. While fir FWD and beech CWD showed only slight insignificant increase.

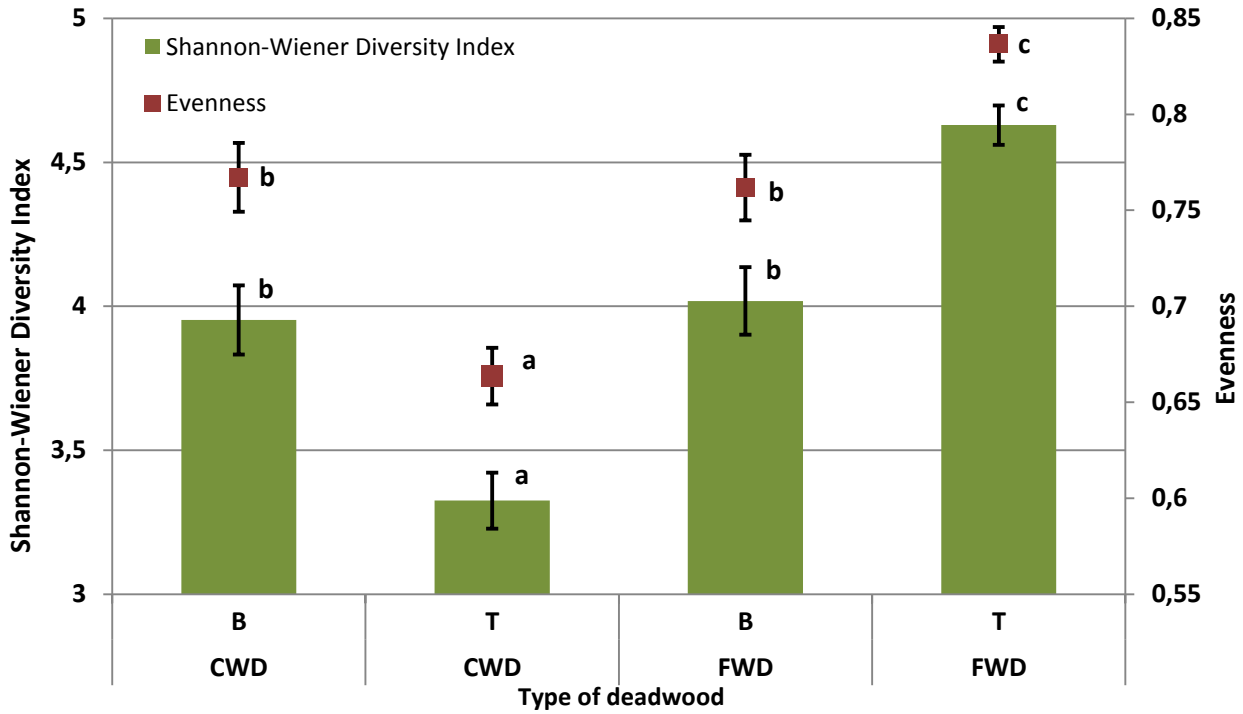


Fig. 24: Values of Shannon-Wiener Diversity Index and Evenness for types of deadwood from year 1 of deadwood experiment. The same resampled sequences were used as for Chao-1 estimate. Standard errors are displayed, statistically significant differences are indicated by different letters (separately for both diversity indices). Note that values of Evenness are on the right axis.

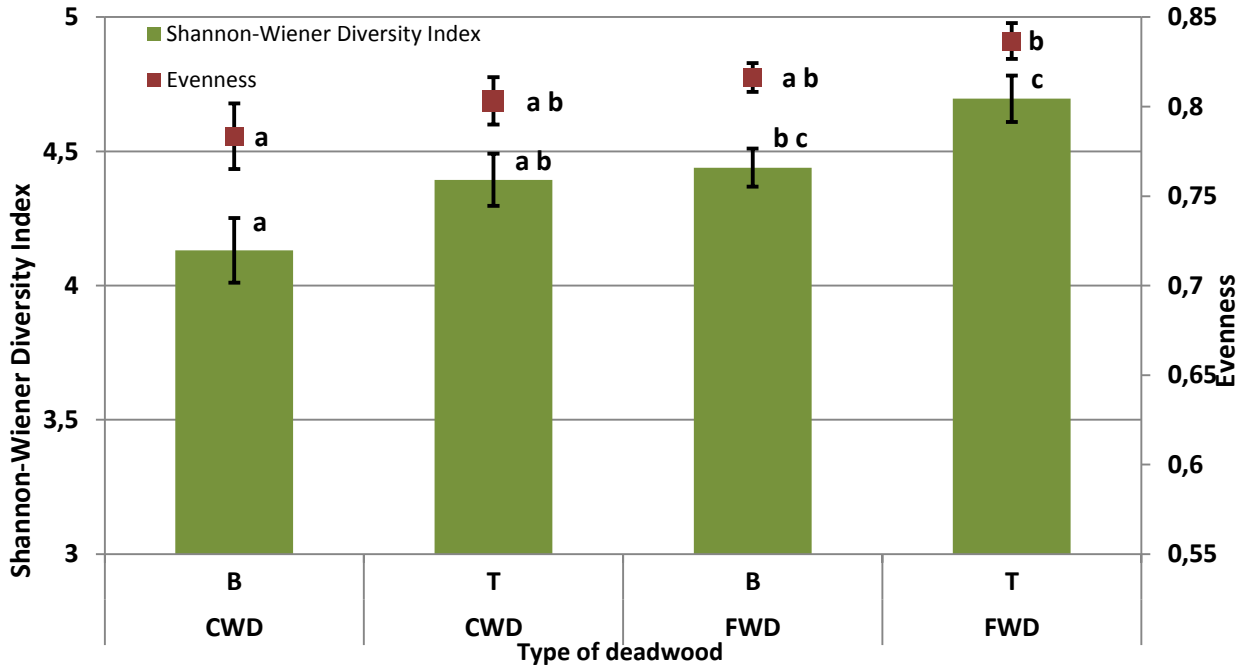


Fig. 25: Values of Shannon-Wiener Diversity Index and Evenness for types of deadwood from year 2 of deadwood experiment. The same resampled sequences were used as for Chao-1 estimate. Standard errors are displayed, statistically significant differences are indicated by different letters (separately for both diversity indices). Note that values of Evenness are on the right axis.

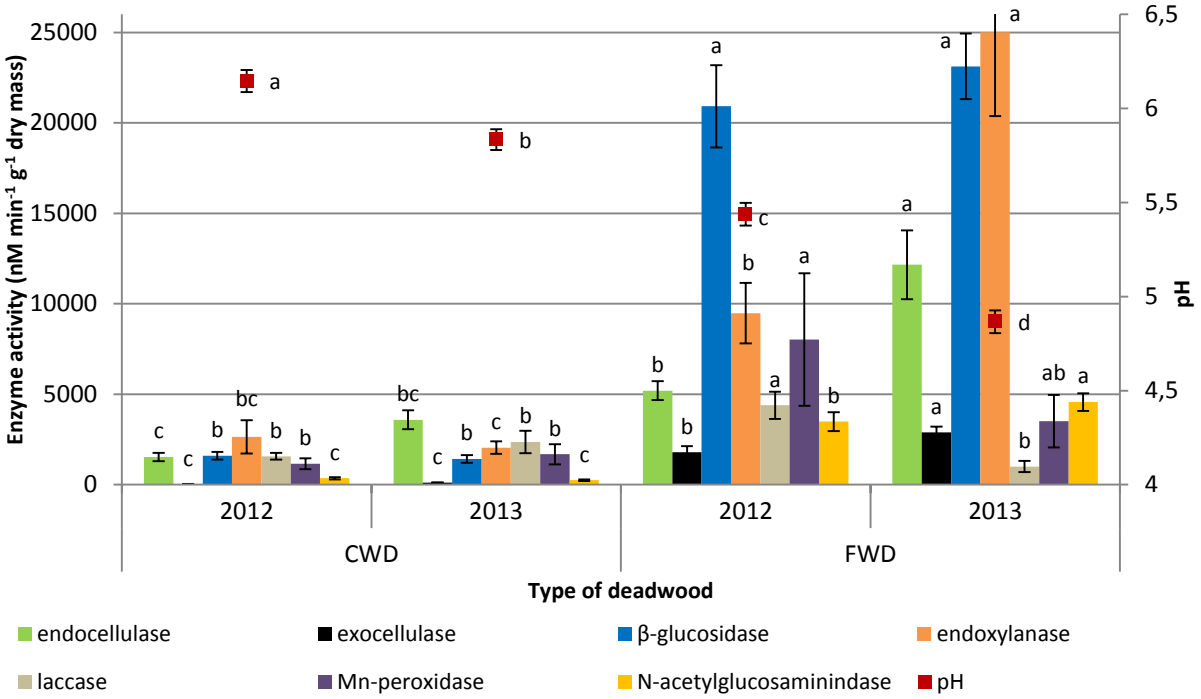
5.2.3 Activities of enzymes produced in deadwood

Measuring of enzyme activities revealed generally higher enzyme acting in FWD then in CWD when comparing one tree species in a single year (Fig. 26A and 26B). This was pronounced in beech FWD in the case of exocellulase (significantly higher activities in both years), β-glucosidase (significantly higher activities in both years), endoxylanase (significantly higher activities in year 2) and Mn-peroxidase (significantly higher activities in year 1). Significantly higher activity in FWD was recorded also for β-glucosidase in fir in both years. *N*-acetylglucosaminidase displayed higher activity in FWD than in CWD for both tree species in both years.

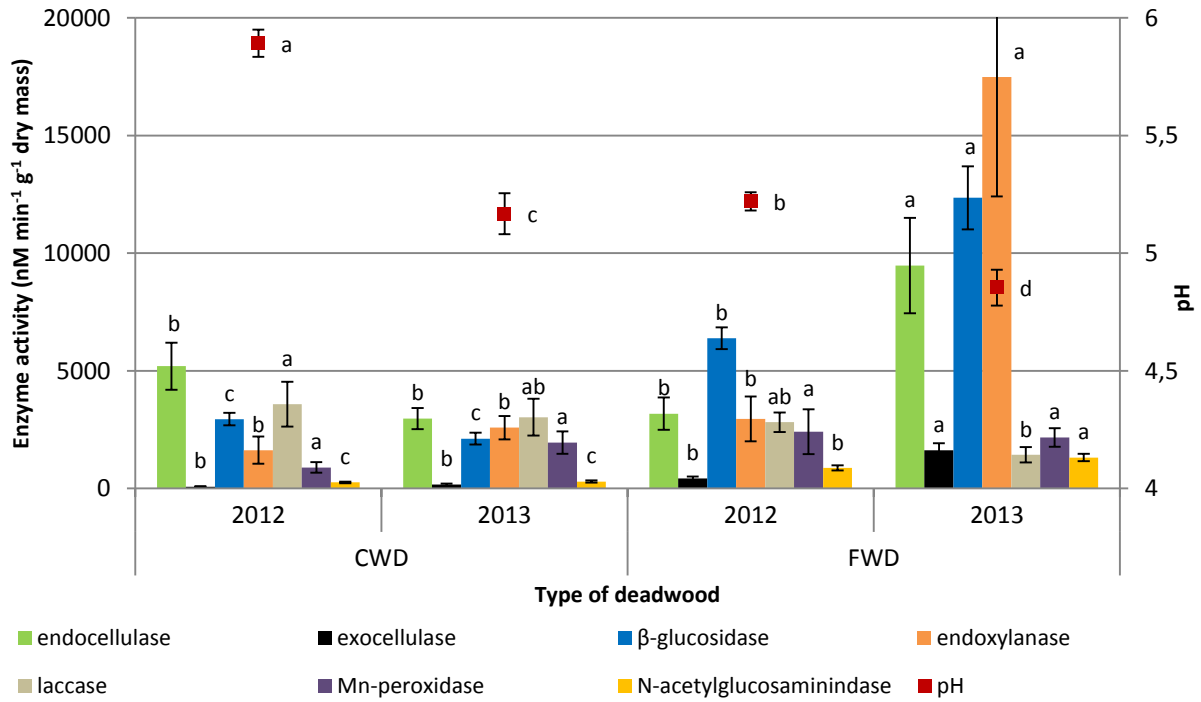
In interseasonal comparison of one tree species and the same deadwood size, increase of activity with time was recorded for endocellulase, exocellulase, endoxylanase and *N*-acetylglucosaminidase in beech FWD. Similarly, endocellulase, exocellulase, β-glucosidase, endoxylanase and *N*-acetylglucosaminidase showed significant increase in activity with time in fir FWD. Laccase show decrease of activity in beech FWD from year 1 to 2 and insignificant decrease of activity with time in CWD and FWD of fir.

Values of pH were significantly lower in FWD of both tree species showing branches as more acidic substrate and logs as more alkaline. In the first year, pH of all deadwood types was significantly different among all treatments. There was decrease in pH in all types of deadwood from year 1 to year 2; pH of beech and fir FWD was similar in year 2. All types except beech CWD showed decrease in content of C. Decrease of N content was also detected in all types except fir FWD where percentage of nitrogen remained the same in year 2. Based on ergosterol content, significantly higher fungal biomass, except of fir deadwood in year 1, was recorded in FWD of both tree species and in year 2 compared to year 1 (Fig. 26C).

A


Enzyme activity (nM min-1 g-1 dry mass)
pH
2012
2013
2012
2013
CWD
FWD
Type of deadwood
endocellulase
exocellulase
β-glucosidase
endoxylanase
laccase
Mn-peroxidase
N-acetylglucosaminindase
pH

B


Enzyme activity (nM min-1 g-1 dry mass)
pH
2012
2013
2012
2013
CWD
FWD
Type of deadwood
endocellulase
exocellulase
β-glucosidase
endoxylanase
laccase
Mn-peroxidase
N-acetylglucosaminindase
pH

C

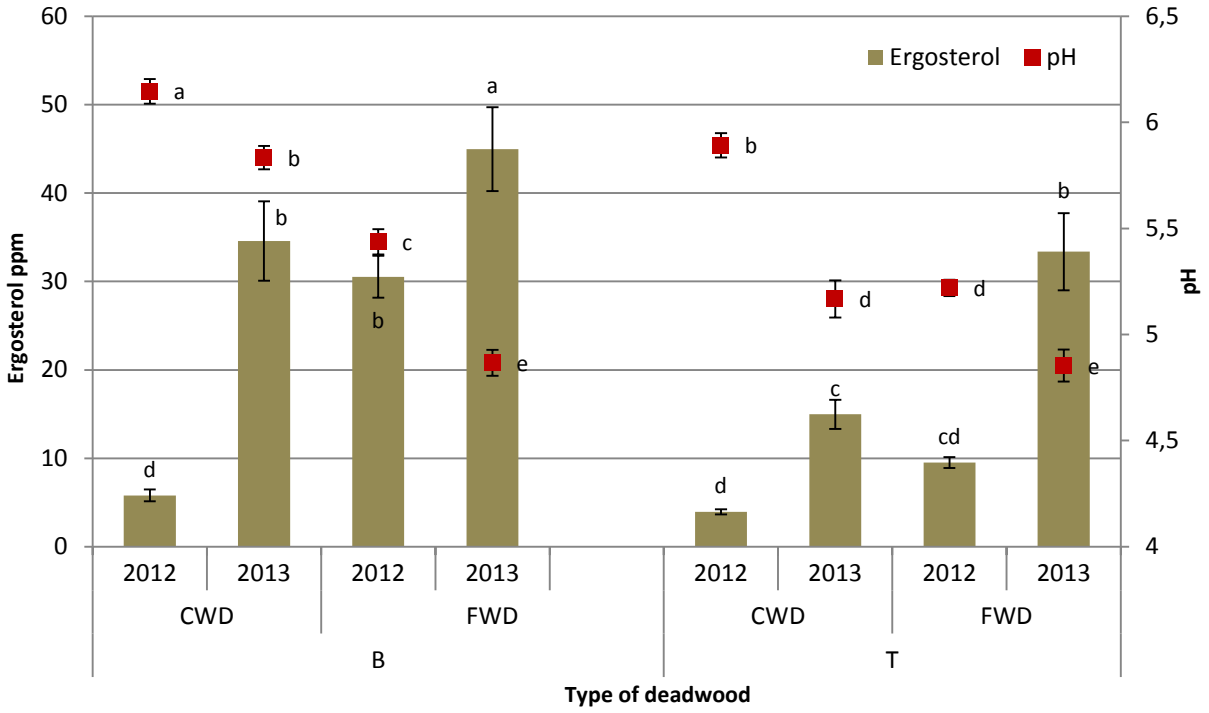


Fig. 26: Activities of extracellular enzymes and pH from different types of deadwood after the first and the second year of decomposition. The data represent means and standard errors. **A)** enzyme activities in beech deadwood; **B)** enzyme activities in fir deadwood; **C)** fungal biomass. Statistically significant differences are indicated by different letters. Note that values of pH in all three graphs are on the right axis.

6 Discussion

6.1 Bacterial succession on *Quercus petraea* leaves and litter

The bacterial phylum *Bacteroidetes* and the proteobacterial classes *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* were detected as the most abundant on *Q. petraea* litter which is consistent with the results obtained for the whole litter horizon of the study site (López-Mondéjar et al., 2015). The bacterial genera *Mucilaginibacter*, *Pedobacter*, *Ferruginibacter*, *Bradyrhizobium*, *Luteibacter*, *Pseudomonas*, *Novosphingobium* and *Burkholderia* were consistently detected as abundant during two years of litter decomposition on the top of forest floor in litterbags as well as in the whole litter horizon (López-Mondéjar et al., 2015). The genera *Mucilaginibacter*, *Pedobacter* and *Ferruginibacter* have putative cellulolytic activity and their abundances were significantly higher in autumn and winter, i.e. early after the litterfall period (López-Mondéjar et al., 2015). The abundance of *Pedobacter* peaked in month 4 which roughly corresponds to the winter. *Mucilaginibacter* and *Ferruginibacter* had their maximum in month 24. According to this, *Pedobacter* preferentially occurs at the phase of early decomposition after leaf senescence, while *Mucilaginibacter* and *Ferruginibacter* are more frequent during advanced decay.

Experiment comparing bacterial community composition in litter horizons under various trees revealed, for oak litter, these abundant genera: *Mucilaginibacter*, *Burkholderia, Pedobacter*, *Pseudomonas*, *Bradyrhizobium Sphingomonas*, *Duganella*, *Janthinobacterium, Hymenobacter* and *Erwinia* (Urbanová et al., 2015). Only the last three genera were infrequent in the results in this thesis, possibly due to higher pH on the post-mining sites where the study was conducted. Together, data confirm that genera *Mucilaginibacter*, *Burkholderia, Pedobacter*, *Pseudomonas* and *Bradyrhizobium* are widely distributed on *Q. petraea* leaf litter.

López-Mondéjar et al., (2015) described higher activities of β-glucosidase, β-xylosidase, endocellulase, exocellulase and *N*-acetylglucosaminidase for whole litter horizon in autumn and winter. This is in agreement with higher enzyme activities

measured at the beginning of two years of leaf litter decomposition (Fig. 15). Elevated enzyme activities during this period are likely response to new input of senescent leaves after abscission. Significant dry mass loss during 4 months of leaf litter decomposition (16.4%, until the end of winter, Fig. 15) is obvious consequence of higher enzymatic activity. Generally, microbial communities after litter input seem to be very active and undergo rapid growth which is reflected in their higher biomass (Purahong et al., 2014).

During the two years of litter decomposition, enzyme production by microbial community reflected substrate depletion and availability (Šnajdr et al., 2011). Decomposition was hence initialized with hemicellulose degradation (until month 4) which was followed by cellulose removal (months 4-12) and late phase was characterized by high activity of ligninolytic enzymes (month 24). According to the Mantel test, activities of cellulolytic enzymes and also laccase may have reflected the composition of bacterial OTUs in separate months. It suggests that bacteria are also active producers of these enzyme. To further connect bacterial community with results provided by enzyme activities, abundances of dominant genera were explored in the terms of their occurrence throughout decomposition. Based on their decreasing prevalence, genera abundant in early phase (month 2) can be considered as typical for initial decomposition or as early colonizers specialized in the degradation of more labile compounds, available at the beginning of decomposition. *r*-selected strategy can help in this period characterized by uneven and not diversified community dominated by a few groups. Another typical pattern during the early phase is fast emergence of early colonizer taxa and than their gradual decrease.

Sampling of live leaves (month -2) revealed unique bacterial community in the phyllosphere which can deliver features advantageous for plant (Innerebner et al., 2011). The genera *Pseudomonas*, *Sphingopyxis*, *Methylosinus*, *Acinetobacter* and *Duganella* dominated on live leaves during pre-senescent phase. Sum of the abundances of early colonizers was decreasing later in time and already in month 2, bacterial community composition differed substantially from the community at month -2. While phyllosphere fungi displayed diverse community and significant part of fungal taxa persisted until month 4 (Voříšková & Baldrian, 2013), our data confirmed partial replacement of taxa dominant in the phyllosphere by groups

abundant in decomposition. Therefore, it can be assumed that phyllosphere bacterial community is adapted to specific conditions on a surface or in an inner part of green leaves (Redford & Fierer, 2009) and does not persist long until subsequent decomposition. Using the primers focusing bacterial 16S rRNA, many sequences from oak chloroplasts were obtained in months -2 and 0. Later months were without these sequences. This illustrates the fast ongoing litter decay.

According to the data about microbial biomass during *Q. petraea* litter decomposition, fungi quantitatively dominated over bacteria in samples from senescent phase (month 0) (Šnajdr et al., 2011) simultaneously, the lowest diversity was observed for fungi in this month. After senescence bacterial biomass increased, while fungal biomass peaked in month 2 and than decreased towards stabilized values until the end of the experiment. This caused a decrease of fungal:bacterial ratio described also during another 2-years experiment with decomposing litter of *Calamagrostis*, *Salix* and *Betula+Populus* (Urbanová et al., 2014). Low amounts of bacterial biomass and specific community composition on live and senescent leaves support the hypothesis that bacteria in the phyllosphere are under strong selective pressure caused by plant itself (Redford et al., 2010) and also by the presence of fungi. Predominance of fungi can provide advantage to these organisms during the following decomposition (Mille-Lindblom et al., 2006; Voříšková & Baldrian, 2013). On the other side, high amount of fungal biomass (peaked in month 2) represents potential substrate for mycolytic bacteria. Dominance of fungal biomass over bacteria was observed in litter from other forests in Central Europe and similarly as in this thesis, bacterial biomass increased during litterbag experiment lasting more than 15 months (Purahong et al., 2014). This confirms bacteria as quantitatively lesser represented organisms which have, however, a potential to succeed during later decomposition.

During middle phase of the decomposition (months 4-8 for bacteria) cellulolytic genera *Pedobacter* and *Sphingomonas* reached their maximal abundances whereas other cellulolytic groups were still increasing. These changes were likely caused by the depletion of labile substrates in leaf litter and higher importance of cellulose as a carbon source. However, this depletion did not restrict increasing bacterial biomass (Šnajdr et al., 2011). PLFA analysis was used as a proxy for change in the structure of

bacterial community (Šnajdr et al., 2011). These results indicated a peak of biomass of *Actinobacteria* in month 12 which was not supported by the sequencing data: *Actinobacteria* were not among the dominant phyla comprising only 2.5% of the total community and no significant peak of abundance. This discrepancy is probably due to the low resolution of analysis based on PLFA. The genera *Duganella* and *Frigoribacterium* which peaked during the middle phase of decomposition (14.9% in month 4 and 3.2% in month 8, respectively) were also among the most abundant in another study focusing decomposition of litter of annual plants (Matulich et al., 2015). However, another major genera from the study of Matulich et al., (2015), the *Curtobacterium* and *Kineococcus*, exhibited only low abundances in *Q. petraea* litter, possibly due to the differences in pH between the litters.

The late phase of *Q. petraea* litter decomposition (months 10-24) was dominated by taxa with increasing prevalence during the two years. Except *Pseudomonas*, *Pedobacter*, *Mucilaginibacter* and *Luteibacter*, dominant genera were different from those with high abundances in the beginning of decomposition. At this time only recalcitrant plant substrates should remain as suggested by predominant lignin degradation during the late decomposition. Bacteria had to (i) successfully degrade these compounds or (ii) find another source of carbon. The first can be achieved by *K*-selected strategy of species, i.e. the strategy of specialized taxa with low growth rates, specialized enzymes and the ability to modulate neighborhood via secondary metabolites. The latter can be achieved by focusing on the mycelial biomass of fungi either in a parasitic or saprotrophic way. The community from late stage of decomposition can carry specialized metabolic pathways which in sum contribute to efficient substrate utilization (Wohl et al., 2004).

Significantly the highest richness of the bacterial community was found in the last months (10-24) of the study, suggesting that this late, nutrient-limited stage is rich in different taxa with complex relationships. In contrast, richness of fungal community did not change significantly after its maximum in month 4. However, composition of fungi underwent successive development as well as bacteria. For development of fungi, rapid changes in dominant taxa throughout the decomposition were characteristic (Voříšková & Baldrian, 2013).

Presented results describe the successive changes in the bacterial community composition as a response to changing conditions in leaf litter during two years of decomposition. Obtained results allowed to describe the elusive phyllosphere community and further to distinguish phases of litter decay characterized by presence of specific taxa and by patterns of their occurrence, most likely caused by the changes in substrate availability.

6.2 Bacterial community inhabiting deadwood

Character of deadwood in the means of accumulated nutrients and fixed carbon differs from that of the leaf litter. Structure of wood and its physico-chemical properties which enable growth and support of a canopy are responsible for rigid nature of this material. Thus, after senescence of a tree, its biomass represents a challenging substrate for saprotrophic organisms. Wood decomposition represents one of the key processes in the nutrient cycle in temperate forests. Input of organic matter in the form of deadwood is not occurring on the annual basis as in the case of leaf litter. This together with more recalcitrant structure contribute to several different principles and strategies during colonization and decomposition.

Tree trunks can be characterized by their mechanical properties which allow rough classification of how much decomposition advanced since the senescence of a tree. Cross-section can be helpful for this assessment as older logs gain elliptical shape due to structural weakness, gravity and following collapse of the trunk (Fraver et al., 2013). Another handy technique allows the estimation of the level of deterioration by measuring wood density (Shorohova & Kapitsa, 2014). Hence, structure changes are tightly connected with the process of decomposition. Although deadwood experiment in this thesis was too short for manifestation of such marked changes, the results still revealed the importance of deadwood size (diameter) on bacterial community structure in the first year. Than, in the second year factors of deadwood size and tree species were balanced. Recently, tree species was identified as an important factor affecting bacterial community composition in beech and spruce deadwood (Hoppe et al., 2015). Moreover, the study of Hoppe et al., (2015) was focused on different decomposition stages of deadwood allowing the monitoring of a longer time

perspective. During it, effect of tree species can be even more pronounced than during two years of experiment in this thesis. Based on the result from the year 1 it can be speculated that deadwood size is of high importance for decomposition and the composition of bacterial community. The bacterial ability (oftenly restricted as the ability of unicellular organisms) to colonize substrate in this initial phase is important. Although some taxa are known to be adapted as colonizers (Clausen, 1996) not all of them probably succeed in invading into the wood especially into its inner parts. These need the initial disruption carried out by stronger colonizers from either bacterial or fungal groups. And so it is crucial for these saprotrophs if colonizers occurs on branches with lower volume or thick logs. This is in opposite with data about fungal community from the same samples (Zrůstová, 2014). For fungal community in the year 1, tree species was more important than diameter. Together these results confirm bacteria as generalists and oppositely fungi as tree-specific decomposers with faster exploitation of substrate.

Weakening of the effect of deadwood size and higher tree species importance in the year 2 can be explained by gradual colonization and ongoing changes in the chemical properties which reflect the tree species according to its original characteristics. pH, carbon and nitrogen content are among the most important parameters of substrate chemistry. Bacteria are sensitive to pH changes due to narrow ranges for optimal growth (Rousk et al., 2010) and the importance of pH on community composition was also confirmed in this study.

The decreasing C:N ratio in wood (Bray et al., 2012; Hoppe et al., 2015; Rajala et al., 2011) which is the consequence of CO_2 efflux and relative nitrogen accumulation in substrate and microbial biomass makes wood more habitable as decomposition proceeds. The initially high C:N ratio causes environment very hostile and thus prevents wood colonization by several taxa. Indeed, percentage of carbon in all deadwood types from Bavaria except beech CWD decreased significantly with time. However, nitrogen percentage showed also decrease in deadwood except fir FWD. Probably, experiment was held at very short time scale so pattern of nitrogen accumulation could not be observed as it may be pronounced during advanced decay.

In accordance with the common pattern of wood acidification due to metabolic activity of saprotrophic fungi (De Boer et al., 2005), samples of wood showed

significantly lower pH in the second year. Moreover, FWD was more acidic than CWD which can be accounted to the more advanced decomposition in deadwood with lower diameter and the activity of fungi that acidify substrate through the production of organic acids such as oxalic acid (Clausen, 1996). Higher decomposition rates in FWD are also supported by higher activities of enzymes acting on cellulose, cellobiose, hemicellulose and lignin in FWD.

Fungal community in wood undergoes its specific development. Biomass of fungi was higher in FWD of both tree species and as supposed there was increase in fungal biomass in the year 2. This well correlates with lower pH of FWD. As a consequence of the fungal community development and senescence of fungal mycelia, higher enzyme activity of *N*-acetylglucosaminidase in FWD in the year 2 was observed. The enzyme degrades fungal biomass and it is known to be produced by mycophagous bacteria enabling feeding on chitin as a convenient source of nutrients. For example, the recorded genera *Paenibacillus* and *Myxococcus* from the group myxobacteria were previosuly described as mycophagous (Dijksterhuis et al., 1999; Homma, 1984) and showed high relative abundances in year 2 in fir FWD while another mycophagous genus *Collimonas* (De Boer et al., 2004) was more abundant in beech FWD. Valášková et al., (2009) identified the bacterial strain from the genus *Sphingomonas*, the strain from the genus *Rahnella* and several strains from the genus *Burkholderia* to be able to utilize *N*-acetyl-D-glucosamine (monomer of chitin) as a growth substrate. These genera were among the most abundant in deadwood and the genus *Burkholderia* was significantly more abundant in FWD, irrespective to the tree species and year, than in CWD.

Frequency of sampling was lower in deadwood experiment in comparison with leaf litter decomposition from the first part of the thesis due to the much slower rates of deadwood turnover. Thus, it was not possible to observe peaks of enzyme activities and distinguish individual waves of enzyme production at finer resolution as was described in the two years of leaf litter decomposition. Community potential for degradation of cellulose can be estimated according to activities of endocellulases and exocellulases – enzymes directly interacting with cellulose fibrils. Genes for β-glucosidases are probably widespread also in noncellulolytic taxa (Berlemont & Martiny, 2013). Therefore, their activity, which is often high (Sinsabaugh et al.,

2008), does not sufficiently indicate cellulose degradation. According to this, FWD showed higher rates of cellulose decomposition when compared with CWD despite the high β-glucosidase activity in the latter.

FWD also harboured significantly more diverse communities of bacteria in the second year compared to CWD. Because of their better accessibility and faster decomposition, FWD can host more diverse bacterial community which showed diversity increase between years. Deadwood in this study were in the cotact with ground which can facilitate colonization by soil or litter bacteria. For branches this colonization can be even more faster than in the case of logs. Oppositely, logs can offer more niches and represent objects with bigger surface area thus stochastically receiving more air-borne microorganisms or their spores. These latter factors are driving fungal community (Bässler et al., 2010), however, according to presented results they do not favour logs for bacterial colonization.

Communities can be further compared not only by their diversity but also by similarity in composition of OTUs. Communities in branches and logs of beech were similar to each other so this tree species host beech-specific OTUs. On the other hand, branches and logs of fir hosted different communities with fir CWD being more similar to beech CWD and with fir FWD being more similar to beech FWD.

Recent study focusing bacterial community in beech and spruce deadwood of different decomposition stages (Hoppe et al., 2015) described bacterial phyla *Actinobacteria* and *Firmicutes* as decreasing with time in both beech and spruce, phylum *Bacteroidetes* as decreasing only in spruce and bacterial class *Gammaproteobacteria* as decreasing in beech. However, similar pattern was not recorded in experiment from Bavaria. Likely, shorter time period of deadwood experiment did not allow manifestation of compositional changes at higher taxonomic level. Distributions of selected abundant bacterial genera or genera with interesting decomposition activities showed differences not only between deadwood types but also in interseasonal comparison. Genera with previously recorded cellulolytic capability *Mucilaginibacter*, *Burkholderia*, *Dyella* and *Luteibacter* (López-Mondéjar et al., 2015; Štursová et al., 2012) shared pattern of higher abundances in FWD supporting the hypothesis about diversified community in rapidly decomposing branches. However, not all cellulolytic genera displayed the same pattern. Data about

Mucilaginibacter and *Burkholderia* from leaf litter experiment in the first part of the thesis suggests their adaptation to late decomposition phases and they can play similar role in deadwood, particularly in FWD. *Sphingomonas* as the most prevalent genus despite its cellulolytic and lignin-modifying activity did not show differences between deadwood types which suggests its general affinity to decomposing wood. Methanotrophic genus *Methylovirgula* was identified as the most abundant bacterial OTU in deadwood of beech and spruce (Hoppe et al., 2015). Interestingly, this genus was not recorded in this study. Another methanotrophic genera abundant in the study of Hoppe et al., (2015) were *Methylocystis* and *Methylocella* that were also observed in some samples from both trees and years. *Pedobacter* as a genus involved in early leaf litter decomposition showed preference for FWD and beech CWD. Moreover, performing mycobag experiment, *Pedobacter* was recognized as mycophagous together with *Variovorax* (Brabcová – personal communication). *Variovorax* also showed significantly higher abundance in beech FWD – result consistent with other mycophagous taxa and higher amount of fungal biomass in FWD.

The litter layer which cover forest floor and underlying soil can be inhabited by diverse bacterial community represented also by taxa with a potential to degrade plant polymers (Berlemont & Martiny, 2013; Šturs ová et al., 2012; Tian et al., 2014). Soil and older litter can serve as reservoirs for bacteria and fungi colonizing deadwood which is in contact with ground. This contact enables faster decomposition of fallen trees when compared with standing snags (Shorohova & Kapitsa, 2014). In this way, litter layer and lying deadwood are tightly linked. In comparison, initially colonized deadwood and leaf litter habitat display similar abundant bacterial genera. The genus *Pseudomonas* was recorded as dominant in deadwood and its occurrence during leaf litter decomposition was the highest in the early phase (and even in the phyllosphere community). The genus *Sphingomonas* has putative cellulolytic and lignin-modifying activity. It was the most abundant genus in deadwood at all and displayed maximum of abundance in the middle phase of leaf litter decomposition (the time of rapid cellulose utilization). Similarly *Pedobacter*, it was abundant in deadwood and peaked in the middle phase of leaf litter decomposition. *Mucilaginibacter* and *Burkholderia* from advanced litter-decay were another abundant

taxa in deadwood. Consistence in abundant taxa from deadwood and leaf litter implies their similar traits in these habitats.

Experiment focusing bacterial community structure throughout initial two years of deadwood decay which was described in this thesis, provided some insight into the microenvironment of wood as the substrate where factors such as complexity of material, its chemical differences and direct interactions between inhabiting microorganisms are key drivers of bacterial community structure itself. Instead of being static, whole community is determined by the size of deadwood. Later determination by chemical and structural changes in substrate occurs. This is caused by nature of deadwood (its species) and activity of other saprotrophes including fungi. When considering the rate of decomposition, it is higher for branches as supposed from higher enzyme activities and more diversified community encompassing members with tools for primary degradation of plant polymers (Wohl et al., 2004). As decomposition proceeds total diversity of community increases and traits of present microorganisms change from first colonizers towards specialists adapted for later climax phases. Thus, resembling much more rapid leaf litter decomposition.

7 Conclusions

Different successional phases of bacteria on leaf litter can be distinguished. Richness of community increases with time.

Live leaves are inhabited by unique bacterial community. Dominant taxa of phyllosphere community are replaced after senescence of leaves.

Different life strategies can be traced when comparing early comunity composition and late community composition. Some groups showed specific pattern of occurrence according to their ecology.

Bacterial abundances are driven by the changes in substrate availability.

Size of decomposing deadwood affects most the composition of early bacterial community. Tree species is important later as decomposition proceeds.

This can be consequence of restricted bacterial ability to colonize rigid wood substrate.

Bacterial community composition inhabiting wood is driven by other factors than fungal community composition.

Branches from later stages of decomposition harbour more diverse communities and exhibit higher activities of cellulolytic enzymes.

Some cellulolytic and lignin-modifying genera are more abundant in branches than in logs.

Despite different nature of leaf litter and deadwood, two years of decomposition showed generally similar pattern of successional course with comparable abundant taxa and pointing of community to diverse advanced phase. Although this process is much slower in persistent deadwood than in the leaf litter.

8 References

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9 Appendix

Cluster title (n=sequences for consensus)	Best hit (accession number)	E value	Similarity (%)	Relative abundance in months (%) ± SE									
				-2	0	2	4	6	8	10	12	18	24
|CL00000000002|n=19305	*Sphingomonas (HE814648)*	1,44E-128	99.6	0.8 ± 0.3	2.3 ± 1.4	5.5 ± 1.8	9.8 ± 2.1	10.5 ± 1.6	12.6 ± 2.7	3.6 ± 1.2	2.8 ± 0.2	1.6 ± 0.5	0.2 ± 0.0
|CL00000000003|n=18458	*Pseudomonas (KC865279)*	8,59E-131	100	20.6 ± 4.7	15.3 ± 9.2	16.8 ± 5.0	8.4 ± 1.2	3.4 ± 0.4	0.9 ± 0.2	0.4 ± 0.1	5.1 ± 3.5	1.5 ± 0.3	0.9 ± 0.2
|CL00000000005|n=9846	*Luteibacter (AB627008)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 0.3	2.0 ± 0.6	3.6 ± 1.0	3.8 ± 0.8	2.0 ± 0.9	4.8 ± 0.8	6.8 ± 2.4	0.7 ± 0.1
|CL00000000008|n=8032	*Mucilaginibacter (EU423302)*	3,99E-129	99.6	0.2 ± 0.1	0.1 ± 0.0	0.4 ± 0.2	0.7 ± 0.2	3.4 ± 1.0	2.4 ± 0.4	3.7 ± 0.7	3.8 ± 0.1	4.5 ± 1.2	10.8 ± 1.3
|CL00000000011|n=6108	*Paucibacter (JX949591)*	8,59E-131	100	0.7 ± 0.4	0.8 ± 0.4	0.4 ± 0.2	0.2 ± 0.0	0.8 ± 0.1	1.2 ± 0.2	3.5 ± 0.6	2.5 ± 0.3	4.4 ± 0.8	6.9 ± 0.4
|CL00000000012|n=5174	*Burkholderia (AM392324)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.8 ± 0.3	1.6 ± 0.3	2.5 ± 1.0	2.8 ± 1.1	2.7 ± 0.5	2.0 ± 0.2	4.4 ± 0.5
|CL00000000001|n=30568	*Pedobacter (HE814666)*	3,99E-129	99.6	0.0 ± 0.0	0.0 ± 0.0	16.3 ± 3.5	27.3 ± 2.9	20.7 ± 1.5	8.4 ± 2.7	2.7 ± 0.5	0.8 ± 0.1	1.5 ± 0.7	0.4 ± 0.1
|CL00000000017|n=4097	*Frigoribacterium (JQ977229)*	8,59E-131	100	1.6 ± 0.9	1.9 ± 0.0	0.2 ± 0.1	0.9 ± 0.2	2.4 ± 0.4	3.1 ± 0.9	1.6 ± 0.4	0.8 ± 0.3	0.9 ± 0.3	0.3 ± 0.1
|CL00000000004|n=17264	*Duganella (JF778667)*	8,59E-131	100	5.7 ± 3.3	8.1 ± 5.2	13.7 ± 1.2	14.7 ± 0.7	6.0 ± 0.6	1.7 ± 0.7	0.9 ± 0.2	0.9 ± 0.5	0.2 ± 0.1	0.1 ± 0.0
|CL00000000018|n=3574	*Mucilaginibacter (AM490403)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	1.8 ± 0.3	1.2 ± 0.3	1.0 ± 0.3	1.3 ± 0.1	1.8 ± 0.5	3.9 ± 0.6
|CL00000000019|n=3280	*Ideonella (KC355350)*	8,59E-131	100	0.1 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	1.2 ± 0.2	1.7 ± 0.2	1.8 ± 0.3	1.2 ± 0.3	1.9 ± 0.4	1.2 ± 0.2
|CL00000000014|n=4497	*Rhizobium (KC853173)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.4	2.0 ± 0.3	3.4 ± 0.3	2.6 ± 0.4	1.3 ± 0.3	0.7 ± 0.2	0.6 ± 0.1	0.2 ± 0.0
|CL00000000009|n=7133	*Sphingomonas (EF467848)*	1,88E-117	97.6	1.5 ± 1.2	3.8 ± 2.2	0.9 ± 0.5	2.0 ± 0.6	4.0 ± 0.9	7.0 ± 3.5	1.3 ± 0.6	1.2 ± 0.6	0.4 ± 0.1	0.1 ± 0.0
|CL00000000007|n=8144	*Massilia (JF496419)*	1,86E-127	99.2	0.0 ± 0.0	0.0 ± 0.0	7.7 ± 1.3	5.0 ± 0.9	3.4 ± 0.5	1.1 ± 0.3	2.0 ± 1.2	0.5 ± 0.1	0.3 ± 0.2	0.0 ± 0.0
|CL00000000020|n=2948	*Bacillus (JQ437583)*	3,99E-129	99.6	0.0 ± 0.0	0.7 ± 0.3	0.3 ± 0.1	0.2 ± 0.0	1.4 ± 0.1	2.7 ± 0.5	1.5 ± 0.3	1.4 ± 0.3	0.5 ± 0.2	0.1 ± 0.0
|CL00000000034|n=1573	*Acidobacterium (AM086241)*	1,87E-122	98	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	1.0 ± 0.2	1.2 ± 0.2	0.5 ± 0.2	1.4 ± 0.2
|CL00000000016|n=4190	*Bradyrhizobium (HQ694740)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	1.3 ± 0.8	2.2 ± 0.3	2.8 ± 0.5	3.2 ± 0.5	3.5 ± 0.8
|CL00000000022|n=2820	unclass. genus (NR_108115.1)	4,17E-99	92.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.7 ± 0.3	1.6 ± 0.3	2.1 ± 0.3	1.8 ± 0.5	2.0 ± 0.6
|CL00000000023|n=2414	*Sphingomonas (AB744218)*	8,59E-131	100	0.0 ± 0.0	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	1.5 ± 0.4	1.9 ± 0.3	0.7 ± 0.2	0.9 ± 0.1	0.4 ± 0.2	0.1 ± 0.0
|CL00000000030|n=1755	*Mucilaginibacter (JN590349)*	1,86E-127	99.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	1.2 ± 0.3	1.8 ± 0.3
|CL00000000015|n=4335	*Janthinobacterium (GU213418)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	5.5 ± 0.8	1.5 ± 0.3	0.7 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.2
|CL00000000025|n=2139	*Sphingomonas (KC748362)*	1,86E-127	99.2	0.0 ± 0.0	0.4 ± 0.2	0.2 ± 0.2	0.6 ± 0.1	1.5 ± 0.2	1.6 ± 0.4	0.7 ± 0.2	0.6 ± 0.2	0.3 ± 0.1	0.0 ± 0.0
|CL00000000029|n=1859	*Burkholderia (JF763852)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.4	1.7 ± 1.1	0.7 ± 0.2	1.2 ± 0.4	0.5 ± 0.1
|CL00000000043|n=1106	*Granulicella (FR716684)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.5 ± 0.2	0.7 ± 0.1
|CL00000000010|n=6334	*Pseudomonas (KC790237)*	8,59E-131	100	0.5 ± 0.3	0.2 ± 0.2	5.9 ± 3.2	6.0 ± 1.1	2.3 ± 0.2	0.6 ± 0.4	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
|CL00000000024|n=2152	*Novosphingobium (KC435022)*	3,99E-129	99.6	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.2	0.6 ± 0.1	1.5 ± 0.3	1.9 ± 0.4	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0
|CL00000000026|n=2106	*Bradyrhizobium (JN221510)*	1,86E-127	99.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.8 ± 0.1	1.4 ± 0.1	1.0 ± 0.4	1.1 ± 0.2	0.7 ± 0.3	0.1 ± 0.0
|CL00000000031|n=1698	*Granulicella (HQ687090)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.3 ± 0.1	0.7 ± 0.2	1.3 ± 0.3	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.1 ± 0.1
|CL00000000032|n=1656	unclass. genus (AB081581)	8,89E-106	94.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.2	1.2 ± 0.5	1.4 ± 0.3	1.2 ± 0.2	0.6 ± 0.2
|CL00000000044|n=1083	*Herbaspirillum (AY367037)*	3,99E-129	99.6	1.2 ± 1.2	0.5 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.2
|CL00000000035|n=1464	*Mucilaginibacter (JX268541)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	1.1 ± 0.2	0.8 ± 0.2	0.2 ± 0.1	0.6 ± 0.1	0.4 ± 0.2	0.7 ± 0.3
|CL00000000045|n=1007	unclass. genus (AF491884)	3,99E-129	99.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.1 ± 0.1
|CL00000000038|n=1303	*Beijerinckia (FR874230)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.9 ± 0.3	0.7 ± 0.1	0.8 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
|CL00000000053|n=825	*Mucilaginibacter (AB681894)*	8,71E-121	98.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	1.4 ± 0.3
|CL00000000036|n=1399	*Sphingomonas (KC987002)*	8,59E-131	100	0.0 ± 0.0	0.6 ± 0.3	0.8 ± 0.3	1.5 ± 0.6	0.5 ± 0.2	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000040|n=1174	*Luteibacter (KC355343)*	8,65E-126	98.8	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.2	0.7 ± 0.2	1.6 ± 0.1
|CL00000000041|n=1152	*Burkholderia (AM392350)*	8,59E-131	100	0.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000050|n=900	*Phenylobacterium (AM411916)*	4,14E-104	94.5	0.5 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.6 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000042|n=1125	*Jahnella (GU207876)*	9,15E-86	90.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.7 ± 0.0	0.5 ± 0.1	1.3 ± 0.4	0.0 ± 0.0
|CL00000000046|n=984	*Sphingomonas (JQ977431)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.6 ± 0.0	0.8 ± 0.3	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
|CL00000000049|n=928	*Devosia (JX950045)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.8 ± 0.1	0.5 ± 0.0	0.8 ± 0.2	0.3 ± 0.1
|CL00000000039|n=1294	*Massilia (JX950006)*	8,59E-131	100	1.4 ± 1.0	1.6 ± 1.4	1.6 ± 0.6	0.7 ± 0.3	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000055|n=800	*Stenotrophomonas (EU075218)*	1,90E-112	96	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.2	0.3 ± 0.1
|CL00000000056|n=787	*Granulicella (KC924939)*	8,71E-121	97.6	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
|CL00000000027|n=1995	*Rahnella (KC351183)*	8,59E-131	100	0.7 ± 0.5	0.1 ± 0.0	3.0 ± 2.2	0.7 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
|CL00000000033|n=1581	*Methylosinus (AJ458477)*	8,83E-111	95.3	6.9 ± 1.5	10.6 ± 4.6	0.1 ± 0.1	0.6 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000037|n=1312	*Acinetobacter (KC920980)*	8,59E-131	100	4.2 ± 0.9	1.4 ± 0.7	2.2 ± 1.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000052|n=849	*Mucilaginibacter (JQ955678)*	8,65E-126	99.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	1.1 ± 0.1
|CL00000000063|n=648	*Mycobacterium (FR693307)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.5 ± 0.4	0.9 ± 0.2
|CL00000000048|n=936	*Gluconacetobacter (AB627120)*	1,90E-112	96.4	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.5 ± 0.0	0.9 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
|CL00000000051|n=894	*Phenylobacterium (AM411916)*	8,83E-111	96	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	1.0 ± 0.1	0.5 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
|CL00000000068|n=604	unclass. genus (AF491884)	1,88E-117	96.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.3 ± 0.1
|CL00000000060|n=682	*Azospirillum (HE801967)*	9,02E-96	93.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	0.3 ± 0.0	0.7 ± 0.1	0.4 ± 0.2	0.1 ± 0.0
|CL00000000062|n=651	*Pedobacter (KC888981)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.3	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.8 ± 0.1
|CL00000000066|n=633	*Acidisphaera (AF376024)*	3,99E-129	99.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.2 ± 0.2
|CL00000000067|n=605	*Mucilaginibacter (FN400860)*	3,99E-129	99.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	1.0 ± 0.1
|CL00000000070|n=590	*Acidisphaera (AF376024)*	1,90E-112	96.4	1.3 ± 0.5	4.5 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000074|n=525	*Chthoniobacter (AY388649)*	7,33E-62	86.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.8 ± 0.3	0.4 ± 0.1	0.2 ± 0.1	0.5 ± 0.1
|CL00000000075|n=515	*Haliangium (CP001804)*	4,23E-89	91.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.1 ± 0.0
|CL00000000115|n=316	*Acinetobacter (KF020733)*	8,59E-131	100	1.6 ± 0.6	1.1 ± 0.8	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000125|n=287	*Acidiphilium (KC924950)*	1,93E-102	94.5	1.0 ± 0.1	1.9 ± 1.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

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Cluster title (n=sequences for consensus)	Best hit (accession number)	E value	Similarity (%)	Relative abundance in months (%) ± SE									
				-2	0	2	4	6	8	10	12	18	24
|CL00000000065|n=647	*Mucilaginibacter (AM933506)*	8,71E-121	98	0.2 ± 0.2	4.4 ± 1.9	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000069|n=601	*Variovorax (AB509388)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.0
|CL00000000085|n=438	*Asticcacaulis (HE814760)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.2
|CL00000000110|n=327	*Frateuria (JQ726640)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.5 ± 0.1
|CL00000000120|n=302	*Flavobacterium (KC330349)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.5 ± 0.3
|CL00000000142|n=241	*Oleomonas (AJ784808)*	1,19E-79	89.1	0.7 ± 0.5	1.9 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000210|n=142	*Hymenobacter (JX841069)*	8,65E-126	98.8	0.8 ± 0.5	1.4 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000059|n=682	*Sphingomonas (KC493207)*	8,59E-131	100	0.3 ± 0.0	1.2 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.1 ± 0.0
|CL00000000064|n=647	*Granulicella (HQ687087)*	1,88E-117	97.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
|CL00000000071|n=583	*Pseudomonas (KC139458)*	8,59E-131	100	0.2 ± 0.2	0.0 ± 0.0	0.7 ± 0.4	0.3 ± 0.2	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000072|n=550	*Pseudomonas (AB819482)*	4,14E-104	93.7	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.0
|CL00000000077|n=484	*Pedobacter (JQ977410)*	8,59E-131	100	0.2 ± 0.1	0.0 ± 0.0	0.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.3	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.0 ± 0.0
|CL00000000078|n=468	*Sediminibacterium (KC505149)*	1,86E-127	99.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.2 ± 0.1
|CL00000000079|n=464	*Phenylobacterium (KC492989)*	4,08E-114	96.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.4 ± 0.2	0.3 ± 0.0	0.4 ± 0.1	0.1 ± 0.0
|CL00000000081|n=459	unclass. genus (AB081581)	8,89E-106	94.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
|CL00000000082|n=459	*Sphingomonas (EU784670)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000083|n=444	*Chitinophaga (JF710262)*	3,99E-129	99.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	1.4 ± 0.6	0.1 ± 0.0
|CL00000000084|n=443	*Erwinia (AM048801)*	8,59E-131	100	1.1 ± 0.3	0.0 ± 0.0	0.6 ± 0.6	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
|CL00000000086|n=435	*Ferruginibacter (KC690141)*	1,88E-117	96.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	0.5 ± 0.1	0.2 ± 0.1
|CL00000000088|n=421	*Chitinophaga (KF228163)*	5,24E-118	96.8	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.1
|CL00000000089|n=408	*Acidocella (KC662252)*	8,71E-121	98	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0	0.0 ± 0.0
|CL00000000099|n=370	*Mucilaginibacter (AB267719)*	8,65E-126	98.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.5 ± 0.3	0.5 ± 0.1
|CL00000000100|n=364	*Mucilaginibacter (AB681894)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.0
|CL00000000102|n=357	*Pedobacter (JQ977531)*	8,65E-126	99.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.5 ± 0.1
|CL00000000158|n=212	*Sphingomonas (AY081166)*	8,59E-131	100	0.6 ± 0.4	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000178|n=177	*Hymenobacter (JX949243)*	1,87E-122	98	0.0 ± 0.0	1.6 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000194|n=160	*Mycobacterium (JX469393)*	8,59E-131	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.1
|CL00000000198|n=155	*Hymenobacter (JN090860)*	8,71E-121	98	0.9 ± 0.7	1.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000224|n=134	*Herbaspirillum (KF170816)*	8,59E-131	100	1.3 ± 0.6	0.3 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000243|n=119	*Ferruginibacter (KC690141)*	1,87E-122	98.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.5 ± 0.1
|CL00000000265|n=108	*Comamonas (GQ140333)*	8,59E-131	100	0.3 ± 0.3	0.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000294|n=96	unclass. genus (DQ059300)	9,41E-66	85.8	0.6 ± 0.4	1.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000344|n=76	*Anaeromyxobacter (AB795400)*	4,25E-89	90.6	1.3 ± 1.1	0.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
|CL00000000362|n=70	*Methylosinus (FR798965)*	8,89E-106	94.1	0.5 ± 0.4	0.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table S1: Abundant bacterial OTUs detected in leaf litter experiment (section 5.1.1). Displayed OTUs showed abundances at least three times ≥ 0.5% in all samples. OTUs are sorted in descending order according to counts of samples with abundances over this threshold. Data represent values from BLASTn, means of relative abundances and standard errors from samples belonging to different months.

Genus	Relative abundance in months (%) ± SE									
	-2	0	2	4	6	8	10	12	18	24
Pseudomonas	27.9 ± 1.4	15.9 ± 9.0	24.3 ± 9.0	15.2 ± 1.6	5.9 ± 0.4	1.9 ± 0.6	1.1 ± 0.2	6.2 ± 3.5	2.3 ± 0.4	1.5 ± 0.2
Sphingomonas	3.7 ± 1.5	10.1 ± 0.4	8.7 ± 3.0	14.9 ± 2.1	20.0 ± 2.1	26.1 ± 5.8	7.9 ± 2.5	7.2 ± 1.1	3.7 ± 0.9	0.7 ± 0.0
Mucilaginibacter	0.4 ± 0.3	4.5 ± 2.0	1.5 ± 0.6	1.7 ± 0.4	8.1 ± 1.6	6.2 ± 1.3	7.7 ± 0.9	9.1 ± 0.4	10.9 ± 2.1	22.7 ± 2
Granulicella	0.2 ± 0.1	1.7 ± 0.8	0.7 ± 0.4	0.9 ± 0.2	2.3 ± 0.3	4.0 ± 0.3	3.1 ± 0.2	2.8 ± 0.4	1.8 ± 0.5	2.0 ± 0.2
Burkholderia	0.1 ± 0.1	0.4 ± 0.2	1.0 ± 0.6	2.4 ± 0.5	2.9 ± 0.6	4.0 ± 1.3	5.3 ± 2.2	4.0 ± 0.7	4.0 ± 0.6	5.5 ± 0.6
Luteibacter	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 0.4	2.1 ± 0.6	3.9 ± 1.0	4.4 ± 0.9	2.4 ± 0.9	5.4 ± 0.8	7.7 ± 2.6	2.3 ± 0.2
Pedobacter	0.3 ± 0.1	0.1 ± 0.0	17.5 ± 4.2	28.2 ± 2.7	21.8 ± 1.8	8.9 ± 2.8	3.2 ± 0.5	1.5 ± 0.3	2.9 ± 1.1	2.4 ± 0.3
Duganella	5.7 ± 3.3	8.1 ± 5.2	13.9 ± 1.2	14.9 ± 0.7	6.1 ± 0.7	1.7 ± 0.7	1.2 ± 0.2	1.0 ± 0.5	0.4 ± 0.2	0.2 ± 0.0
Paucibacter	0.7 ± 0.4	0.8 ± 0.4	0.4 ± 0.2	0.2 ± 0.0	0.8 ± 0.1	1.2 ± 0.2	3.5 ± 0.6	2.5 ± 0.3	4.5 ± 0.8	7.0 ± 0.4
Phenylobacterium	0.5 ± 0.2	0.5 ± 0.4	0.4 ± 0.3	0.7 ± 0.1	1.1 ± 0.2	2.2 ± 0.2	2.2 ± 0.5	1.5 ± 0.2	1.0 ± 0.1	0.4 ± 0.0
Frigoribacterium	1.7 ± 1.0	2.0 ± 0.1	0.2 ± 0.1	1.0 ± 0.2	2.5 ± 0.4	3.2 ± 0.9	1.6 ± 0.4	0.8 ± 0.4	1.0 ± 0.3	0.3 ± 0.1
Massilia	2.5 ± 1.4	2.2 ± 1.8	9.7 ± 1.7	6.1 ± 0.9	3.7 ± 0.4	1.1 ± 0.3	2.0 ± 1.2	0.7 ± 0.1	0.5 ± 0.2	0.2 ± 0.0
Ideonella	0.1 ± 0.1	0.1 ± 0.0	0.5 ± 0.2	0.3 ± 0.0	1.2 ± 0.2	1.7 ± 0.2	1.8 ± 0.3	1.3 ± 0.3	2.0 ± 0.4	1.2 ± 0.2
Bradyrhizobium	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	1.0 ± 0.1	2.8 ± 0.7	3.3 ± 0.4	4.1 ± 0.6	4.1 ± 0.6	3.7 ± 0.9
Rhizobium	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.5	2.1 ± 0.4	3.5 ± 0.4	2.7 ± 0.4	1.5 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	0.3 ± 0.1
Acidobacterium	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.8 ± 0.2	1.0 ± 0.1	1.4 ± 0.2	1.7 ± 0.2	0.8 ± 0.3	1.8 ± 0.3
Beijerinckia	0.0 ± 0.0	0.2 ± 0.2	0.2 ± 0.2	1.0 ± 0.3	0.8 ± 0.1	1.1 ± 0.3	0.6 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
Bacillus	0.2 ± 0.1	0.8 ± 0.3	0.3 ± 0.1	0.2 ± 0.0	1.4 ± 0.1	2.8 ± 0.5	1.5 ± 0.3	1.5 ± 0.3	0.5 ± 0.2	0.1 ± 0.0
Novosphingobium	0.0 ± 0.0	0.2 ± 0.2	0.4 ± 0.3	0.7 ± 0.0	1.8 ± 0.3	2.2 ± 0.4	0.8 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0
Azospirillum	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.6 ± 0.2	1.1 ± 0.2	1.6 ± 0.3	1.4 ± 0.3	0.8 ± 0.2
Herbaspirillum	2.6 ± 1.9	0.8 ± 0.4	0.4 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.1
Chitinophaga	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	1.4 ± 0.4	1.5 ± 0.2	3.5 ± 0.9	2.2 ± 0.3
Chthoniobacter	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.8 ± 0.3	4.1 ± 0.4	2.4 ± 0.4	1.8 ± 0.2	2.3 ± 0.3
Janthinobacterium	0.0 ± 0.0	0.0 ± 0.0	5.8 ± 0.8	1.7 ± 0.4	0.8 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.2
Steroidobacter	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	0.8 ± 0.1	1.1 ± 0.3	1.1 ± 0.3	0.6 ± 0.1
Gluconacetobacter	0.5 ± 0.1	0.8 ± 0.5	0.2 ± 0.1	0.3 ± 0.1	0.6 ± 0.0	1.0 ± 0.2	0.2 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
Prosthecobacter	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	0.4 ± 0.1	1.3 ± 0.1
Devosia	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.9 ± 0.1	0.6 ± 0.0	1.6 ± 0.7	0.4 ± 0.1
Acidisphaera	1.3 ± 0.5	4.6 ± 0.8	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.2 ± 0.2
Haliangium	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.5 ± 0.2	1.1 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.2 ± 0.1
Jahnella	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	1.5 ± 0.5	0.0 ± 0.0
Flavobacterium	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.4 ± 0.2	0.7 ± 0.2	1.0 ± 0.4
Sediminibacterium	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.1	0.8 ± 0.3	1.1 ± 0.1	0.4 ± 0.1
Stenotrophomonas	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	0.5 ± 0.0	0.7 ± 0.2	0.7 ± 0.2	0.4 ± 0.1
Ferruginibacter	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.5 ± 0.3	0.8 ± 0.1	1.5 ± 0.3
Mycobacterium	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	0.6 ± 0.5	2.1 ± 0.2
Acinetobacter	6.0 ± 1.4	2.6 ± 0.2	2.3 ± 1.7	0.3 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
Terrimonas	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.3 ± 0.1
Caulobacter	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	1.6 ± 1.0	0.2 ± 0.0
Gemmatimonas	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.8 ± 0.2	0.4 ± 0.1
Chondromyces	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	0.1 ± 0.0
Chryseolinea	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1	0.8 ± 0.3	0.6 ± 0.2	0.2 ± 0.1
Methylosinus	7.6 ± 1.2	11.4 ± 4.9	0.1 ± 0.1	0.6 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Opitutus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.7 ± 0.1
Rahnella	0.7 ± 0.5	0.1 ± 0.0	3.0 ± 2.2	0.7 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
Rhodoplanes	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.7 ± 0.2	0.8 ± 0.1
Variovorax	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	0.6 ± 0.0
Acidiphilium	1.0 ± 0.1	2.4 ± 1.5	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Hymenobacter	1.7 ± 0.4	5.6 ± 2.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Bdellovibrio	0.4 ± 0.4	0.4 ± 0.4	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Candidatus Xiphinematobacter	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.0
Frateuria	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± .0	0.0 ± 0.0	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.6 ± 0.1
Legionella	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.1
Sphingobacterium	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Asticcacaulis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.8 ± 0.2
Erwinia	1.2 ± 0.3	0.0 ± 0.0	0.7 ± 0.6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Oleomonas	0.7 ± 0.5	1.9 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Acidocella	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	1.1 ± 0.3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Anaeromyxobacter	1.3 ± 1.1	0.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Brevundimonas	1.6 ± 1.6	0.8 ± 0.6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.2	0.3 ± 0.0	0.2 ± 0.1
Comamonas	0.3 ± 0.3	0.8 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Conexibacter	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	0.5 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
Cytophaga	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.9 ± 0.3
Gemmata	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
Methylobacterium	0.5 ± 0.5	1.3 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Reyranella	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
Sphingobium	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.6 ± 0.3	0.2 ± 0.1
unclass. genus	1.0 ± 0.5	1.6 ± 0.8	0.1 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	2.5 ± 1.2	6.7 ± 1.5	8.5 ± 0.8	7.3 ± 1.3	6.0 ± 1.0

Table S2: Bacterial genera detected in leaf litter experiment (section 5.1.1). Displayed genera showed abundances at least three times ≥ 0.5% in all samples. Genera are sorted in descending order according to counts of samples with abundances over this threshold. Data represent means of relative abundances and standard errors from samples belonging to different months.

Cluster title (n=seq. for consensus)	Best hit (accession number)	E value	Similarity (%)	Relative abundance (%) ± SE							
				B				T			
				CWD		FWD		CWD		FWD	
C000001|n=66211	*Frigoribacterium (JQ977229)*	8.78E-131	100	4.3 ± 0.8	b	5.1 ± 0.8	a	2.6 ± 0.4	b	3.9 ± 0.5	b
C000000|n=136621	*Rahnella (KC351183)*	8.78E-131	100	6.9 ± 1.9	b	3.1 ± 0.6	bc	26.3 ± 2.1	a	2.7 ± 1.0	c
C000009|n=25490	*Bradyrhizobium (JN221510)*	8.78E-131	100	1.1 ± 0.2	c	2.4 ± 0.3	a	1.8 ± 0.2	b	1.1 ± 0.2	bc
C000011|n=20894	*Rhizobium (KC853173)*	8.78E-131	100	1.1 ± 0.3	b	1.8 ± 0.3	ab	1.4 ± 0.3	ab	1.8 ± 0.2	a
C000010|n=23035	*Sphingomonas (KC735149)*	4.08E-129	99.6	3.0 ± 0.6	a	2.3 ± 0.3	a	0.6 ± 0.1	c	1.2 ± 0.1	b
C000004|n=37902	*Luteibacter (AB627008)*	8.78E-131	100	0.9 ± 0.4	c	5.6 ± 1.3	ab	1.6 ± 0.3	bc	2.5 ± 0.3	a
C000006|n=34052	*Burkholderia (KF031512)*	8.78E-131	100	0.6 ± 0.2	b	2.1 ± 0.3	ab	1.3 ± 0.4	b	2.4 ± 0.4	a
C000014|n=16203	*Methylosinus (AJ458477)*	1.94E-112	95.7	3.3 ± 0.6	a	2.2 ± 0.7	a	0.5 ± 0.1	b	1.3 ± 0.2	b
C000007|n=30061	*Cellulomonas (KC337106)*	8.78E-131	100	4.5 ± 1.6	a	2.1 ± 0.4	ab	1.3 ± 0.2	b	0.8 ± 0.2	b
C000005|n=36143	*Pedobacter (HE814666)*	4.08E-129	99.6	3.3 ± 1.2	ab	3.7 ± 1.2	ab	1.0 ± 0.2	b	2.6 ± 0.6	a
C000016|n=14664	*Rubrivivax (AM086242)*	8.78E-131	100	0.5 ± 0.1	b	1.4 ± 0.2	a	0.7 ± 0.1	b	1.0 ± 0.1	a
C000013|n=16791	*Bacillus (JQ437583)*	8.78E-131	100	0.9 ± 0.2	a	1.1 ± 0.2	ab	0.4 ± 0.1	b	1.4 ± 0.1	a
C000012|n=20383	*Sphingomonas (EF467848)*	1.93E-117	97.6	0.5 ± 0.1	b	1.4 ± 0.4	ab	0.9 ± 0.2	b	3.3 ± 0.9	a
C000019|n=13064	*Sphingomonas (AB744218)*	8.78E-131	100	1.0 ± 0.2	ab	1.1 ± 0.2	a	0.3 ± 0.1	c	0.7 ± 0.1	b
C000017|n=13726	*Granulicella (KC924939)*	4.11E-124	99.2	0.7 ± 0.2	b	0.6 ± 0.2	ab	0.5 ± 0.1	b	2.0 ± 0.2	a
C000008|n=27809	*Yersinia (AJ011333)*	8.78E-131	100	1.2 ± 0.3	b	1.0 ± 0.3	b	3.3 ± 0.6	a	0.7 ± 0.2	b
C000020|n=12811	*Acidisphaera (AF376024)*	4.11E-124	98.8	1.6 ± 0.6	a	0.5 ± 0.1	ab	0.5 ± 0.2	b	2.3 ± 0.3	a
C000003|n=43440	*Dyella (AB245367)*	8.78E-131	100	1.6 ± 0.4	ab	7.8 ± 2.7	ab	1.0 ± 0.4	b	3.0 ± 1.0	a
C000027|n=9577	*Methylosinus (AJ458477)*	4.17E-114	96	2.1 ± 0.5	a	0.5 ± 0.2	bc	0.2 ± 0.0	c	1.7 ± 0.3	b
C000015|n=15654	*Pseudomonas (JN167957)*	8.78E-131	100	0.7 ± 0.2	a	1.0 ± 0.2	a	1.0 ± 0.3	a	0.5 ± 0.1	a
C000025|n=10019	*Mucilaginibacter (JX268541)*	8.78E-131	100	0.5 ± 0.1	bc	0.9 ± 0.1	ab	0.3 ± 0.1	c	0.9 ± 0.1	a
C000018|n=13650	*Mucilaginibacter (EU423302)*	4.08E-129	99.6	0.2 ± 0.0	c	0.8 ± 0.2	ab	0.5 ± 0.1	bc	1.0 ± 0.1	a
C000002|n=44396	*Pseudomonas (HF913576)*	8.78E-131	100	0.9 ± 0.5	b	0.8 ± 0.2	b	9.5 ± 2.2	a	0.2 ± 0.0	b
C000022|n=10562	*Erwinia (KC677746)*	8.78E-131	100	0.4 ± 0.2	b	0.9 ± 0.3	b	2.0 ± 0.3	a	0.1 ± 0.1	b
C000044|n=6126	*Novosphingobium (KF145130)*	8.78E-131	100	0.7 ± 0.1	a	0.6 ± 0.1	a	0.3 ± 0.1	b	0.5 ± 0.1	a
C000028|n=9327	*Bradyrhizobium (HQ694740)*	8.78E-131	100	0.4 ± 0.1	b	0.8 ± 0.2	a	0.3 ± 0.1	b	1.0 ± 0.2	a
C000026|n=9914	*Jatrophihabitans (JQ346802)*	1.90E-127	99.2	0.5 ± 0.1	b	0.4 ± 0.1	b	0.2 ± 0.0	b	1.5 ± 0.2	a
C000042|n=6654	*Acidiphilium (KC924950)*	9.16E-101	94.5	0.9 ± 0.2	a	0.8 ± 0.2	a	0.1 ± 0.0	c	0.6 ± 0.1	b
C000033|n=8181	*Amnibacterium (KC251736)*	1.90E-127	99.2	0.8 ± 0.1	a	0.5 ± 0.1	ab	0.2 ± 0.0	b	0.5 ± 0.0	b
C000029|n=8775	*Duganella (JF778667)*	8.78E-131	100	0.9 ± 0.2	ab	1.1 ± 0.4	a	0.3 ± 0.1	b	0.4 ± 0.1	b
C000037|n=7350	*Sphingomonas (EF558729)*	8.78E-131	100	1.0 ± 0.3	a	0.4 ± 0.1	b	0.1 ± 0.0	b	0.6 ± 0.1	b
C000031|n=8363	*Acidisoma (AM947652)*	4.08E-129	99.6	0.3 ± 0.1	b	0.4 ± 0.1	ab	0.4 ± 0.1	ab	0.9 ± 0.2	a
C000039|n=7172	*Beijerinckia (FR874230)*	8.78E-131	100	0.5 ± 0.1	ab	0.7 ± 0.3	ab	0.2 ± 0.0	b	0.7 ± 0.1	a
C000040|n=7140	*Ideonella (KC355350)*	8.78E-131	100	0.3 ± 0.1	b	0.8 ± 0.2	a	0.2 ± 0.0	b	0.8 ± 0.1	a
C000030|n=8774	*Burkholderia (HE814630)*	8.78E-131	100	0.2 ± 0.0	b	0.7 ± 0.2	ab	0.2 ± 0.0	b	1.1 ± 0.3	a
C000034|n=8058	*Sphingomonas (EF467848)*	8.84E-126	99.2	0.3 ± 0.1	b	0.4 ± 0.2	ab	0.4 ± 0.1	ab	0.6 ± 0.1	a
C000046|n=6056	*Curtobacterium (KC810833)*	8.78E-131	100	0.3 ± 0.1	b	0.7 ± 0.2	a	0.3 ± 0.0	b	0.3 ± 0.0	b
C000036|n=7650	*Rhizobium (HF566319)*	8.78E-131	100	0.9 ± 0.3	a	0.8 ± 0.2	ab	0.3 ± 0.1	b	0.1 ± 0.0	ab
C000043|n=6449	unclass. *bacterium (EU476046)*	8.78E-131	100	0.5 ± 0.1	a	0.6 ± 0.2	a	0.5 ± 0.2	a	0.3 ± 0.1	a
C000021|n=12009	*Undibacterium (KC505152)*	4.08E-129	99.6	0.2 ± 0.0	b	0.1 ± 0.0	b	2.8 ± 0.5	a	0.2 ± 0.1	b
C000050|n=5193	*Phenylobacterium (JX949351)*	1.93E-117	96.8	1.1 ± 0.3	a	0.2 ± 0.1	b	0.2 ± 0.0	b	0.5 ± 0.1	b
C000054|n=4483	*Granulicella (FR716684)*	4.08E-129	99.6	0.2 ± 0.1	b	0.2 ± 0.0	b	0.2 ± 0.0	b	0.7 ± 0.1	a
C000061|n=3881	*Methylopila (KC447360)*	1.97E-102	93.3	0.3 ± 0.1	ab	0.2 ± 0.0	bc	0.1 ± 0.0	c	0.7 ± 0.1	a
C000035|n=7956	*Mycobacterium (FR693307)*	8.78E-131	100	0.1 ± 0.0	b	0.3 ± 0.1	b	0.1 ± 0.0	b	1.2 ± 0.1	a
C000041|n=6749	unclass. *Actinobacteria (X68459)*	4.11E-124	98.8	0.5 ± 0.3	a	0.1 ± 0.0	a	0.6 ± 0.3	a	0.9 ± 0.2	a
C000023|n=10317	*Stenotrophomonas (KC790309)*	8.78E-131	100	0.2 ± 0.1	b	0.6 ± 0.3	b	1.9 ± 0.4	a	0.0 ± 0.0	b

Table S3: Bacterial OTUs detected in deadwood experiment in samples from year 1 (section 5.2.1). Relative abundances of displayed OTUs were ≥ 0.5% in at least 30 samples out of 124. OTUs are sorted in descending order according to counts of samples with abundances over this threshold. Data represent values from BLASTn, means of relative abundances and standard errors for given groups of different deadwood. Statistically significant differences are indicated by different letters.

	Relative abundance (%) ± SE							
	B				T			
Genus	CWD		FWD		CWD		FWD	
Sphingomonas	8.7 ± 1.2	a	8.3 ± 0.7	a	3.3 ± 0.5	b	8.6 ± 1.0	a
Bradyrhizobium	1.5 ± 0.2	b	3.3 ± 0.3	a	2.1 ± 0.2	b	2.1 ± 0.2	b
Frigoribacterium	4.3 ± 0.8	ab	5.3 ± 0.8	a	2.6 ± 0.4	b	4.0 ± 0.5	ab
Mucilaginibacter	1.4 ± 0.2	b	3.3 ± 0.4	a	1.3 ± 0.2	b	3.3 ± 0.3	a
Rhizobium	2.5 ± 0.5	ab	3.0 ± 0.4	a	1.9 ± 0.3	b	2.0 ± 0.3	ab
Granulicella	2.2 ± 0.4	b	2.1 ± 0.4	b	1.7 ± 0.3	b	6.5 ± 0.6	a
Methylosinus	5.7 ± 1.0	a	2.8 ± 0.9	b	0.7 ± 0.1	c	3.3 ± 0.4	b
Pseudomonas	2.0 ± 0.6	b	2.4 ± 0.3	b	11.2 ± 2.3	a	1.0 ± 0.2	b
Rahnella	6.9 ± 1.9	b	3.1 ± 0.6	b	26.3 ± 2.1	a	2.7 ± 1.0	b
Burkholderia	0.9 ± 0.2	b	2.9 ± 0.3	a	1.5 ± 0.4	b	3.7 ± 0.5	a
Pedobacter	4.2 ± 1.3	a	4.6 ± 1.2	a	1.5 ± 0.2	b	3.0 ± 0.7	ab
Luteibacter	0.9 ± 0.4	b	5.6 ± 1.3	a	1.6 ± 0.3	b	2.5 ± 0.3	b
Bacillus	1.2 ± 0.3	a	1.2 ± 0.2	a	1.4 ± 0.6	a	1.5 ± 0.1	a
Phenylobacterium	1.6 ± 0.3	a	0.8 ± 0.2	b	0.6 ± 0.1	b	1.9 ± 0.2	a
Cellulomonas	4.6 ± 1.7	a	2.2 ± 0.4	ab	1.3 ± 0.3	b	0.8 ± 0.3	b
Rubrivivax	0.5 ± 0.1	c	1.4 ± 0.2	a	0.7 ± 0.1	bc	1.1 ± 0.1	ab
Acidisphaera	2.1 ± 0.7	ab	1.3 ± 0.4	bc	0.8 ± 0.2	c	3.0 ± 0.4	a
Beijerinckia	1.2 ± 0.2	a	1.0 ± 0.3	a	0.4 ± 0.1	b	1.5 ± 0.1	a
Duganella	1.0 ± 0.2	ab	1.4 ± 0.4	a	0.9 ± 0.2	ab	0.6 ± 0.1	b
Chitinophaga	1.2 ± 0.3	b	0.8 ± 0.3	b	3.8 ± 0.7	a	0.6 ± 0.1	b
Yersinia	1.2 ± 0.3	b	1.0 ± 0.3	b	3.4 ± 0.6	a	0.7 ± 0.3	b
Dyella	1.7 ± 0.4	b	8.1 ± 2.7	a	1.1 ± 0.4	b	3.3 ± 1.0	b
Novosphingobium	0.9 ± 0.1	a	0.9 ± 0.2	a	0.4 ± 0.1	b	0.7 ± 0.1	ab
Acidiphilium	1.2 ± 0.2	a	1.0 ± 0.2	a	0.2 ± 0.0	b	1.0 ± 0.1	a
Acidisoma	0.5 ± 0.1	b	0.5 ± 0.1	b	0.5 ± 0.1	b	1.1 ± 0.2	a
Erwinia	0.4 ± 0.2	bc	0.9 ± 0.3	b	2.1 ± 0.4	a	0.2 ± 0.1	c
Jatrophihabitans	0.5 ± 0.2	b	0.4 ± 0.1	b	0.3 ± 0.0	b	1.7 ± 0.2	a
Conexibacter	0.4 ± 0.1	b	0.3 ± 0.0	b	0.4 ± 0.1	b	0.8 ± 0.1	a
Desulfotomaculum	1.0 ± 0.2	a	0.6 ± 0.2	b	0.1 ± 0.0	c	0.6 ± 0.1	b
Amnibacterium	0.8 ± 0.1	a	0.5 ± 0.1	b	0.2 ± 0.0	b	0.5 ± 0.0	b
Brevundimonas	0.5 ± 0.1	b	0.3 ± 0.0	b	0.3 ± 0.0	b	0.8 ± 0.1	a
unclass. genus	3.4 ± 0.5	a	1.9 ± 0.2	b	2.0 ± 0.4	b	3.4 ± 0.3	a

Table S4: Bacterial genera detected in deadwood experiment – year 1 (section 5.2.1). Relative abundances of displayed genera were ≥ 0.5% in at least 30 samples out of 124. Genera are sorted in descending order according to counts of samples with abundances over this threshold. Data represent means of sum of relative abundances for particular genera and standard errors for given groups of different deadwood. Statistically significant differences are indicated by different letters.

Cluster title (n=seq. for consensus)	Best hit (accession number)	E value	Similarity (%)	Relative abundance (%) ± SE							
				B				T			
				CWD		FWD		CWD		FWD	
C000001|n=66211	*Frigoribacterium (JQ977229)*	8.78E-131	100	6.4 ± 0.9	a	5.1 ± 0.4	ab	3.8 ± 0.5	b	4.8 ± 0.6	ab
C000009|n=25490	*Bradyrhizobium (JN221510)*	8.78E-131	100	1.3 ± 0.2	c	1.9 ± 0.2	ab	2.1 ± 0.2	a	1.6 ± 0.1	bc
C000006|n=34052	*Burkholderia (KF031512)*	8.78E-131	100	2.1 ± 0.4	c	5.5 ± 0.6	a	3.2 ± 0.8	bc	4.2 ± 0.6	ab
C000000|n=136621	*Rahnella (KC351183)*	8.78E-131	100	6.4 ± 1.0	b	2.1 ± 0.4	c	9.4 ± 1.6	a	2.2 ± 0.5	c
C000019|n=13064	*Sphingomonas (AB744218)*	8.78E-131	100	1.2 ± 0.2	a	1.2 ± 0.1	a	0.9 ± 0.1	a	1.0 ± 0.1	a
C000007|n=30061	*Cellulomonas (KC337106)*	8.78E-131	100	3.7 ± 1.0	a	2.4 ± 0.5	ab	2.7 ± 0.5	ab	1.1 ± 0.2	b
C000018|n=13650	*Mucilaginibacter (EU423302)*	4.08E-129	99.6	0.8 ± 0.1	b	2.3 ± 0.3	a	0.9 ± 0.1	b	2.3 ± 0.3	a
C000010|n=23035	*Sphingomonas (KC735149)*	4.08E-129	99.6	1.5 ± 0.3	a	1.2 ± 0.1	ab	0.9 ± 0.2	b	1.5 ± 0.2	ab
C000013|n=16791	*Bacillus (JQ437583)*	8.78E-131	100	1.6 ± 0.3	a	1.9 ± 0.2	a	1.3 ± 0.4	a	1.3 ± 0.2	a
C000008|n=27809	*Yersinia (AJ011333)*	8.78E-131	100	3.0 ± 0.6	a	1.3 ± 0.3	b	3.1 ± 0.7	a	1.0 ± 0.3	b
C000011|n=20894	*Rhizobium (KC853173)*	8.78E-131	100	1.0 ± 0.1	ab	0.9 ± 0.1	ab	0.7 ± 0.1	b	1.2 ± 0.2	a
C000016|n=14664	*Rubrivivax (AM086242)*	8.78E-131	100	1.3 ± 0.2	a	1.3 ± 0.3	a	0.7 ± 0.1	b	0.9 ± 0.1	ab
C000017|n=13726	*Granulicella (KC924939)*	4.11E-124	99.2	0.3 ± 0.0	b	1.1 ± 0.2	a	1.5 ± 0.2	a	1.0 ± 0.1	a
C000004|n=37902	*Luteibacter (AB627008)*	8.78E-131	100	1.5 ± 0.3	b	2.7 ± 0.5	a	0.6 ± 0.1	b	1.5 ± 0.3	b
C000015|n=15654	*Pseudomonas (JN167957)*	8.78E-131	100	1.3 ± 0.2	b	2.5 ± 0.6	a	1.7 ± 0.5	ab	0.7 ± 0.1	b
C000002|n=44396	*Pseudomonas (HF913576)*	8.78E-131	100	4.1 ± 2.3	a	2.4 ± 0.7	a	1.8 ± 0.7	a	1.1 ± 0.2	a
C000005|n=36143	*Pedobacter (HE814666)*	4.08E-129	99.6	1.9 ± 0.4	a	1.9 ± 0.4	a	0.4 ± 0.1	b	1.9 ± 0.6	a
C000026|n=9914	*Jatrophihabitans (JQ346802)*	1.90E-127	99.2	0.5 ± 0.1	ab	0.5 ± 0.1	b	0.9 ± 0.2	a	0.8 ± 0.1	ab
C000014|n=16203	*Methylosinus (AJ458477)*	1.94E-112	95.7	1.4 ± 0.3	a	0.8 ± 0.2	b	1.0 ± 0.2	ab	0.5 ± 0.1	b
C000028|n=9327	*Bradyrhizobium (HQ694740)*	8.78E-131	100	0.2 ± 0.1	c	0.7 ± 0.1	b	1.3 ± 0.2	a	0.8 ± 0.1	b
C000035|n=7956	*Mycobacterium (FR693307)*	8.78E-131	100	0.5 ± 0.1	b	0.4 ± 0.1	b	1.0 ± 0.2	a	0.7 ± 0.1	ab
C000012|n=20383	*Sphingomonas (EF467848)*	1.93E-117	97.6	0.4 ± 0.1	b	1.0 ± 0.2	a	0.4 ± 0.1	b	1.1 ± 0.2	a
C000033|n=8181	*Amnibacterium (KC251736)*	1.90E-127	99.2	1.4 ± 0.3	a	0.6 ± 0.1	b	0.5 ± 0.1	b	0.4 ± 0.1	b
C000003|n=43440	*Dyella (AB245367)*	8.78E-131	100	1.0 ± 0.2	b	3.5 ± 1.0	a	0.9 ± 0.3	b	2.3 ± 0.7	ab
C000031|n=8363	*Acidisoma (AM947652)*	4.08E-129	99.6	0.3 ± 0.1	b	0.8 ± 0.1	a	0.7 ± 0.1	ab	1.0 ± 0.2	a
C000030|n=8774	*Burkholderia (HE814630)*	8.78E-131	100	0.3 ± 0.1	b	1.3 ± 0.2	a	0.2 ± 0.0	b	1.0 ± 0.2	a
C000025|n=10019	*Mucilaginibacter (JX268541)*	8.78E-131	100	0.7 ± 0.1	a	0.8 ± 0.1	a	0.1 ± 0.0	b	0.6 ± 0.2	a
C000038|n=7335	*Gryllotalpicola (JQ864374)*	4.08E-129	99.6	0.6 ± 0.2	a	0.6 ± 0.2	a	0.6 ± 0.2	a	1.1 ± 0.4	a
C000042|n=6654	*Acidiphilium (KC924950)*	9.16E-101	94.5	0.5 ± 0.1	ab	0.7 ± 0.1	a	0.3 ± 0.1	bc	0.3 ± 0.0	c
C000034|n=8058	*Sphingomonas (EF467848)*	8.84E-126	99.2	0.3 ± 0.1	a	0.8 ± 0.3	a	1.1 ± 0.7	a	0.6 ± 0.1	a
C000059|n=4131	*Acidobacterium (AM086241)*	1.91E-122	98	0.2 ± 0.0	b	0.6 ± 0.1	a	0.5 ± 0.1	a	0.5 ± 0.1	a
C000020|n=12811	*Acidisphaera (AF376024)*	4.11E-124	98.8	0.2 ± 0.1	b	0.4 ± 0.1	ab	0.6 ± 0.1	a	0.6 ± 0.1	a
C000052|n=4673	*Gluconacetobacter (AB627120)*	1.94E-112	96.4	0.3 ± 0.1	b	0.8 ± 0.2	a	0.2 ± 0.0	b	0.3 ± 0.0	b
C000097|n=2322	unclass. *Flavobacteria (AF491884)*	4.08E-129	99.6	0.1 ± 0.0	c	0.3 ± 0.1	bc	0.5 ± 0.1	ab	0.6 ± 0.1	a
C000039|n=7172	*Beijerinckia (FR874230)*	8.78E-131	100	0.5 ± 0.2	a	0.6 ± 0.1	a	0.4 ± 0.1	ab	0.2 ± 0.0	b
C000040|n=7140	*Ideonella (KC355350)*	8.78E-131	100	0.2 ± 0.0	b	0.6 ± 0.2	a	0.2 ± 0.0	b	0.5 ± 0.1	ab
C000088|n=2634	*Dokdonella (AB245362)*	8.78E-131	100	0.3 ± 0.1	a	0.4 ± 0.1	a	0.4 ± 0.1	a	0.5 ± 0.1	a
C000029|n=8775	*Duganella (JF778667)*	8.78E-131	100	0.9 ± 0.4	a	0.7 ± 0.2	ab	0.1 ± 0.0	b	0.6 ± 0.2	ab
C000021|n=12009	*Undibacterium (KC505152)*	4.083E-129	99.6	0.4 ± 0.1	b	0.1 ± 0.0	b	1.0 ± 0.2	a	0.2 ± 0.1	b
C000085|n=2750	*Rhizomicrobium (AB081581)*	4.23E-104	94.1	0.1 ± 0.0	c	0.3 ± 0.0	b	0.6 ± 0.1	a	0.4 ± 0.1	b

Table S5: Bacterial OTUs detected in deadwood experiment in samples from year 2 (section 5.2.1). Relative abundances of displayed OTUs were ≥ 0.5% in at least 30 samples out of 123. OTUs are sorted in descending order according to counts of samples with abundances over this threshold. Data represent values from BLASTn, means of relative abundances and standard errors for given groups of different deadwood. Statistically significant differences are indicated by different letters.

	Relative abundance (%) ± SE							
	B				T			
Genus	CWD		FWD		CWD		FWD	
Sphingomonas	6.0 ± 1.0	a	6.5 ± 0.6	a	4.8 ± 0.8	a	6.2 ± 0.5	a
Frigoribacterium	6.4 ± 0.9	a	5.1 ± 0.4	ab	3.8 ± 0.5	b	4.9 ± 0.6	ab
Bradyrhizobium	1.5 ± 0.2	c	2.6 ± 0.2	b	3.4 ± 0.2	a	2.4 ± 0.2	b
Mucilaginibacter	2.6 ± 0.3	b	4.8 ± 0.5	a	1.7 ± 0.2	b	4.2 ± 0.4	a
Burkholderia	2.5 ± 0.4	b	7.2 ± 0.7	a	3.4 ± 0.8	b	5.6 ± 0.7	a
Granulicella	1.3 ± 0.2	b	3.4 ± 0.4	a	3.3 ± 0.5	a	3.4 ± 0.3	a
Pseudomonas	6.5 ± 2.9	a	5.9 ± 1.1	a	4.0 ± 0.9	a	2.7 ± 0.4	a
Bacillus	1.7 ± 0.3	a	2.0 ± 0.2	a	1.5 ± 0.4	a	1.7 ± 0.2	a
Rahnella	6.4 ± 1.0	b	2.1 ± 0.4	c	9.4 ± 1.6	a	2.2 ± 0.5	c
Rhizobium	2.5 ± 0.3	a	1.2 ± 0.1	b	1.0 ± 0.1	b	1.3 ± 0.2	b
Cellulomonas	3.8 ± 1.0	a	2.4 ± 0.5	ab	2.8 ± 0.6	ab	1.1 ± 0.2	b
Chitinophaga	2.4 ± 0.7	a	0.9 ± 0.1	b	1.8 ± 0.2	ab	1.1 ± 0.1	b
Yersinia	3.1 ± 0.6	a	1.4 ± 0.3	b	3.1 ± 0.7	a	1.0 ± 0.3	b
Methylosinus	1.7 ± 0.3	a	1.2 ± 0.2	ab	1.2 ± 0.2	ab	0.9 ± 0.1	b
Pedobacter	2.5 ± 0.4	a	2.4 ± 0.5	a	0.6 ± 0.1	b	2.3 ± 0.7	a
Rubrivivax	1.3 ± 0.2	a	1.3 ± 0.3	a	0.7 ± 0.1	b	0.9 ± 0.1	ab
Chthoniobacter	0.5 ± 0.1	c	0.8 ± 0.1	c	1.5 ± 0.2	b	2.1 ± 0.3	a
Luteibacter	1.5 ± 0.3	bc	2.7 ± 0.5	a	0.6 ± 0.1	c	1.6 ± 0.3	b
Conexibacter	0.4 ± 0.1	c	0.5 ± 0.0	c	1.2 ± 0.1	a	0.8 ± 0.1	b
Dyella	1.1 ± 0.2	b	4.2 ± 1.0	a	1.1 ± 0.3	b	3.1 ± 0.7	a
Acidisphaera	0.6 ± 0.1	b	1.0 ± 0.2	a	1.0 ± 0.2	ab	0.8 ± 0.1	ab
Mycobacterium	0.6 ± 0.1	b	0.6 ± 0.1	b	1.1 ± 0.2	a	0.8 ± 0.1	ab
Rhizomicrobium	0.2 ± 0.0	c	0.8 ± 0.1	b	1.4 ± 0.1	a	0.9 ± 0.1	b
Jatrophihabitans	0.6 ± 0.1	a	0.6 ± 0.1	a	0.9 ± 0.2	a	0.9 ± 0.1	a
Acidisoma	0.3 ± 0.1	b	0.8 ± 0.2	a	0.7 ± 0.1	ab	1.0 ± 0.2	a
Phenylobacterium	0.4 ± 0.1	b	0.6 ± 0.1	b	0.9 ± 0.2	b	2.0 ± 0.6	a
Beijerinckia	0.8 ± 0.2	a	0.9 ± 0.1	a	0.6 ± 0.1	a	0.6 ± 0.1	a
Amnibacterium	1.4 ± 0.3	a	0.6 ± 0.1	b	0.5 ± 0.1	b	0.4 ± 0.1	b
Nocardioides	1.1 ± 0.3	a	0.4 ± 0.1	b	1.0 ± 0.2	a	0.5 ± 0.1	b
Acidiphilium	0.7 ± 0.1	ab	0.9 ± 0.2	a	0.4 ± 0.1	bc	0.4 ± 0.1	c
Dokdonella	0.5 ± 0.1	a	0.6 ± 0.1	a	0.5 ± 0.1	a	0.7 ± 0.1	a
Acidobacterium	0.3 ± 0.0	c	0.9 ± 0.1	a	0.5 ± 0.1	bc	0.7 ± 0.1	ab
Duganella	1.0 ± 0.4	a	0.9 ± 0.2	ab	0.3 ± 0.1	b	0.9 ± 0.3	ab
Clostridium	1.2 ± 0.4	a	0.5 ± 0.1	b	1.3 ± 0.3	a	0.5 ± 0.1	b
Gryllotalpicola	0.6 ± 0.2	a	0.6 ± 0.2	a	0.6 ± 0.2	a	1.2 ± 0.4	a
Acidipila	0.1 ± 0.0	c	0.4 ± 0.1	b	1.0 ± 0.2	a	0.6 ± 0.1	b
Flavobacterium	0.6 ± 0.2	ab	0.5 ± 0.1	b	0.2 ± 0.1	b	1.0 ± 0.2	a
Gluconacetobacter	0.5 ± 0.1	b	1.1 ± 0.3	a	0.3 ± 0.1	b	0.4 ± 0.1	b
unclass. genus	3.2 ± 0.3	b	3.1 ± 0.2	b	4.7 ± 0.4	a	4.6 ± 0.3	a

Table S6: Bacterial genera detected in deadwood experiment – year 2 (section 5.2.1). Relative abundances of displayed genera were ≥ 0.5% in at least 30 samples out of 123. Genera are sorted in descending order according to counts of samples with abundances over this threshold. Data represent means of sum of relative abundances for particular genera and standard errors for given groups of different deadwood. Statistically significant differences are indicated by different letters.

A

	OTU - genus	Specificity	Fidelity	Stat
FWD/B 2012	C0144 - *Acidisphaera*	0.63	0.94	0.77
	C0154 - *Streptomyces*	0.73	0.74	0.74
	C0003 - *Dyella*	0.57	0.94	0.73
	C0004 - *Luteibacter*	0.54	0.97	0.73
FWD/B 2013	C0123 - *Terrimonas*	0.63	0.84	0.73
	C0098 - *Rhodopila*	0.57	0.87	0.71
FWD/T 2012	C0051 - *Kineosporia*	0.96	0.97	0.96
	C0064 - *Granulicella*	0.88	1.00	0.94
	C0035 - *Mycobacterium*	0.73	1.00	0.86
	C0115 - *Phenylobacterium*	0.75	0.97	0.86
	C0076 - *Actinoplanes*	0.73	0.97	0.84
	C0181 - *Conexibacter*	0.70	1,00	0.84
	C0190 - *Prosthecobacter*	0.71	0.97	0.83
	C0127 - *Frankia*	0.67	1,00	0.82
	C0139 - *Stenotrophomonas*	0.68	0.97	0.81
	C0082 - *Brevundimonas*	0.66	1.00	0.81
	C0026 - *Jatrophihabitans*	0.65	1.00	0.81
	C0188 - *Acidipila*	0.68	0.94	0.80
	C0197 - *Mycobacterium*	0.76	0.84	0.80
	C0116 - *Granulicella*	0.68	0.94	0.80
	C0199 - *Acidocella*	0.69	0.90	0.79
	C0020 - *Acidisphaera*	0.62	1.00	0.79
	C0061 - *Methylopila*	0.62	1.00	0.79
	C0054 - *Granulicella*	0.61	1.00	0.78
	C0150 - *Methylibium*	0.63	0.97	0.78
	C0017 - *Granulicella*	0.61	1.00	0.78
	C0027 - *Methylosinus*	0.60	1.00	0.77
	C0110 - *Solirubrobacter*	0.58	1.00	0.76
	C0105 - *Derxia*	0.58	1.00	0.76
	C0182 - *Sphingomonas*	0.66	0.87	0.76
	C0114 - *Actinomycetospora*	0.60	0.97	0.76
	C0012 - *Sphingomonas*	0.57	1,00	0.75
	C0138 - *Rhodopila*	0.62	0.87	0.74
	C0080 - *Granulicella*	0.56	0.97	0.74
	C0030 - *Burkholderia*	0.53	1.00	0.73
	C0059 - *Acidobacterium*	0.53	1.00	0.73
	C0145 - *Roseococcus*	0.58	0.90	0.73
	C0149 - *Ferruginibacter*	0.64	0.81	0.72
	C0119 - *Sphingomonas*	0.51	1.00	0.72
	C0100 - *Granulicella*	0.53	0.94	0.71
	C0130 - *Methylocystis*	0.53	0.94	0.71
	C0158 - *Actinomadura*	0.69	0.71	0.70
FWD/T 2013	C0051 - *Kineosporia*	0.89	0.77	0.83
	C0182 - *Sphingomonas*	0.70	0.90	0.80
	C0124 - *Methylacidiphilum*	0.64	0.84	0.73

The corresponding table and description are on the next page.

B

	OTU - genus	Specificity	Fidelity	Stat
CWD/B 2012	C0063 - *Rhizobiales* order	0.69	0.77	0.73
CWD/B 2013	C0036 - *Rhizobium*	0.78	0.94	0.86
	C0043 - NO HIT	0.80	0.87	0.83
	C0099 - *Hansschlegelia*	0.68	0.97	0.81
	C0104 - *Ochrobactrum*	0.98	0.58	0.75
	C0126 - *Ancylobacter*	0.73	0.77	0.75
	C0129 - *Methylobacterium*	0.61	0.87	0.73
	C0022 - *Erwinia*	0.61	0.81	0.70
CWD/T 2012	C0002 - *Pseudomonas*	0.86	1.00	0.93
	C0021 - *Undibacterium*	0.86	1.00	0.93
	C0000 - *Rahnella*	0.71	1.00	0.84
	C0184 - *Herbaspirillum*	0.82	0.84	0.83
	C0032 - *Chitinophaga*	0.77	0.84	0.81
	C0048 - *Chitinophaga*	0.71	0.90	0.80
	C0023 - *Stenotrophomonas*	0.71	0.90	0.80
	C0153 - *Herminiimonas*	0.77	0.84	0.80
	C0022 - *Erwinia*	0.62	1.00	0.79
	C0024 - *Vagococcus*	0.74	0.84	0.79
	C0008 - *Yersinia*	0.57	1.00	0.75
	C0089 - *Pseudomonas*	0.56	0.97	0.74
CWD/T 2013	C0133 - *Acidipila*	0.86	0.93	0.90
	C0185 - *Telmatospirillum*	0.82	0.77	0.79
	C0021 - *Undibacterium*	0.65	0.97	0.79
	C0110 - *Solirubrobacter*	0.60	0.97	0.76
	C0096 - *Conexibacter*	0.59	0.97	0.76
	C0184 - *Herbaspirillum*	0.74	0.77	0.75
	C0024 - *Vagococcus*	0.70	0.80	0.75
	C0143 - *Actinobacteria* class	0.70	0.80	0.75
	C0173 - *Chthoniobacter*	0.58	0.97	0.75
	C0181 - *Conexibacter*	0.57	0.97	0.75
	C0000 - *Rahnella*	0.55	1.00	0.74
	C0085 - *Rhizomicrobium*	0.55	1.00	0.74
	C0028 - *Bradyrhizobium*	0.52	1.00	0.72
	C0172 - *Alcaligenes*	0.75	0.67	0.71
	C0140 - Cand. *Xiphinematobacter*	0.62	0.80	0.70

Table S7: Tables of indicator species for FWD (**A**) and CWD (**B**) from deadwood experiment (section 5.2.1). Values were derived from 200 most abundant OTUs in each year. Only statistically significant hits with Stat value over 0.7 were displayed.